Molecular testing for Lynch syndrome in people with colorectal cancer

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Contributions of authors

Tristan Snowsill	Contributed to the systematic reviews of end-to-end studies and economic evaluations. Contributed to the development, implementation and checking of the independent economic assessment. Provided overall project management. Contributed to the writing and editing of the report.
Helen Coelho	Led the systematic review of diagnostic test accuracy and contributed to all aspects of this review. Contributed to the writing and editing of the report.
Nicola Huxley	Led the review of published cost-effectiveness studies. Contributed to the development, implementation and checking of the independent economic assessment. Contributed to the writing and editing of the report.
Tracey Jones-Hughes	Contributed to all aspects of the diagnostic test accuracy systematic review. Wrote the background section. Contributed to the writing and editing of the report.
Simon Briscoe	Developed and conducted the literature searches for the systematic reviews of diagnostic test accuracy, end-to-end studies and economic evaluations. Contributed to the writing and editing of the report.
Ian Frayling	Advised on current clinical practice and scientific understanding of Lynch syndrome. Contributed to the writing and editing of the report.
Chris Hyde	Led the systematic review of end-to-end studies. Contributed to the writing and editing of the report.

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Abstract

Background

Inherited mutations in DNA mismatch repair (MMR) genes lead to an increased risk of colorectal cancer (CRC), gynaecological cancers and other cancers, known as Lynch syndrome (LS). Risk-reducing interventions can be offered to individuals with known LS-causing mutations. The mutations can be identified by comprehensive testing of the MMR genes, but this would be prohibitively expensive to do in the general population. Tumour-based tests – microsatellite instability (MSI) and MMR immunohistochemistry (IHC) – may be used in CRC patients to identify individuals at high risk of LS for genetic testing. *MLH1* promoter hypermethylation and *BRAF* V600E testing can also be conducted on tumour material to rule out certain sporadic cancers.

Methods

Systematic reviews were conducted of the published literature for diagnostic test accuracy studies of MSI and/or IHC for LS, end-to-end studies of screening for LS in CRC patients, and economic evaluations of screening for LS in CRC patients.

A model-based economic evaluation was also conducted to extrapolate long-term outcomes from the results of the diagnostic test accuracy review. The model was created by extending a model previously developed by the authors.

Results

Ten studies were identified which evaluated the diagnostic test accuracy of MSI and/or IHC for identifying LS in CRC patients. For MSI, estimates of sensitivity ranged from 66.7% to 100.0%, and estimates of specificity from 61.1% to 92.5%. For IHC, estimates of sensitivity ranged from 80.8% to 100.0%, and estimates of specificity from 80.5% to 91.9%. When tumours showing low levels of MSI are treated as “test positive” results, the sensitivity of MSI testing increases but specificity falls (a threshold effect).

No end-to-end studies of screening for LS in CRC patients were identified.

Nine economic evaluations of screening for LS in CRC were identified. One of these was a cost–utility analysis conducted in the UK previously published by the authors of this report. None of the included studies fully matched the decision problem, and hence a new economic evaluation was required.

The base case results in the economic evaluation suggest that screening for LS in CRC patients using IHC, *BRAF* V600E and *MLH1* methylation testing would be cost-effective at a threshold of £20,000 per quality-adjusted life year (QALY). The incremental cost-effectiveness ratio for this strategy is £11,008 per QALY compared to no screening. Screening without tumour tests is not predicted to be cost-effective (more costly and less effective than another strategy).

Conclusions

There is evidence from a systematic review that MSI and IHC can be used to identify LS in CRC patients, although there is heterogeneity in the methods and results of the studies identified. There is no high-quality empirical evidence that screening for LS in CRC patients improves long-term outcomes, and so an evidence linkage approach using modelling is necessary. Key determinants of whether screening is cost-effective are: diagnostic performance of tumour-based tests; risk of CRC without surveillance; number of relatives identified for cascade testing; effectiveness of colonoscopic surveillance; acceptance of genetic testing.

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Executive summary

Background

Lynch syndrome is the most common form of genetically-defined, hereditary colorectal cancer, accounting for approximately 3.3% of colorectal tumours. An estimated 175,000 people in the UK have Lynch syndrome and this leads to over 1,100 colorectal cancers per year across the UK.

As an autosomal dominant disorder, if one parent has Lynch Syndrome, there is a 50% chance that each of their children will inherit it. Although characterised by an increased risk of colorectal cancer, individuals with Lynch syndrome are also at increased risk of other cancers such as endometrial, ovarian, stomach, small intestine, hepatobiliary tract, urinary tract, brain and skin.

Lynch syndrome is caused by constitutional pathogenic mutations in DNA mismatch repair (MMR) genes. The five genes known to be involved are: MutL homolog 1 (*MLH1*), MutS homologs 2 and 6 (*MSH2*, *MSH6*), Postmeiotic segregation increased 2 (*PMS2*) and Epithelial cell adhesion molecule (*EPCAM*). Mismatch repair proteins are involved in recognising and repairing errors during DNA replication.

Colorectal cancers associated with Lynch syndrome develop at a young age and are believed to arise, in part, from pre-existing discrete proximal colonic adenomas. However, there is evidence to suggest that patients with colorectal cancer from Lynch syndrome families survive longer than sporadic colorectal cancer patients with same-stage tumours, which may be due to a reduced propensity to metastasise.

Individuals with Lynch syndrome have a risk to age 70 of 33–46% of colorectal cancer. This is in contrast to the general population where the risk is 5.5% and 7.3% for women and men, respectively. The average age of colorectal cancer onset is 44 years in members of families that meet the criteria for Lynch syndrome, whereas for the general population it is 60–65 years.

The risk of cancer in people with Lynch syndrome varies according to the affected MMR gene, with mutations in *MLH1* and *MSH2* conferring the highest cancer risk.

Frequent colonoscopy with polypectomy is believed to decrease the mortality of colorectal cancer in patients with Lynch syndrome. The current screening protocol recommended by the Mallorca Group of InSiGHT is for colonoscopy every 1–2 years starting from age 25 years.

Testing for Lynch syndrome in people with colorectal cancer is sometimes targeted using the Amsterdam criteria and Revised Bethesda Guidelines. Both guidelines use criteria mainly based on family cancer history and age at onset. These methods are unlikely to be sensitive enough to detect all patients with Lynch syndrome because family history is not always reliable or available, and some people with Lynch syndrome may not meet all the criteria.

Overall, the majority of colorectal tumours from individuals with Lynch syndrome genes have two distinguishing characteristics and therefore the diagnostic technologies focus on these aspects:

- Microsatellite instability – PCR-based MSI testing as carried out by UKAS-accredited regional genetics laboratories using validated in-house tests. Molecular microsatellite instability testing involves polymerase chain reaction (PCR) amplification of standardised DNA markers from a tumour tissue sample and a matched healthy tissue sample from the same patient. Laboratories may use a panel of 10 or more markers. Instability in 30% or more of the markers is considered MSI-H, less than 30% is considered MSI-L and no shifts or additional peaks is considered MSS.
- Loss of expression of the mismatch repair proteins in tumour cells – MMR immunohistochemistry (IHC) uses antibodies to test for the presence or absence of MMR proteins in tumour cells compared to non-tumour cells. If nuclear staining is abnormal for any MMR protein(s), this suggests the MMR system is affected.

A small proportion of possibly Lynch-related tumours do not exhibit any abnormality on analysis by IHC, even though they have lost MMR function as demonstrated by microsatellite instability (MSI).

Around 10 to 15% of sporadic CRCs show MSI-H and in the vast majority of these, this will be due to acquired promoter methylation of the *MLH1* gene leading to loss of MLH1 protein expression. However, a small proportion of sporadic MSI-H CRCs will occur due to loss of MSH2, MSH6 or PMS2 and germline *MLH1* hypermethylation will be observed in a very small number of some colorectal cancers due to LS. Approximately half of the sporadic CRCs with MSI-H will also have a *BRAF* V600E mutation – this is a specific mutation in the *BRAF* gene which almost never occurs in tumours arising in LS. Therefore, testing for *MLH1* promoter methylation and *BRAF* V600E mutation represent ways of distinguishing sporadic CRC from LS in a proportion of MLH1-negative tumours.

The gold standard for diagnosis of Lynch syndrome is comprehensive screening for constitutional mutations in the MMR genes and *EPCAM*. This screening is conducted using a DNA sequencing method to detect point mutations, small insertions and deletions and MLPA to detect large structural DNA abnormalities, such as genomic deletions, duplications and rearrangements.

Although comprehensive screening for constitutional mutations should accurately detect the majority of known Lynch syndrome-causing mutations, there are some occasions where a novel mutation may be identified which is of uncertain significance. Since such a variant cannot be demonstrated to be pathogenic or non-pathogenic, it is not possible to make a diagnosis or recommendations for management, such as colorectal surveillance.

Value proposition for the technologies under consideration

Individuals with Lynch syndrome-causing mutations have elevated risks of a number of cancers, and in particular colorectal cancer and gynaecological cancers. Due to the elevated risk these cancers will often develop at an earlier age than average.

If individuals can be identified to have a Lynch syndrome-causing mutation then risk-reducing interventions can be offered. These interventions include colonoscopic surveillance (to identify and remove precursor lesions or identify colorectal cancer at an early stage), prophylactic hysterectomy and bilateral salpingo-oophorectomy, aspirin chemoprevention and gynaecological surveillance (although there is no high-quality evidence that gynaecological surveillance is effective).

The prevalence of Lynch syndrome-causing mutations in the general population is low (estimated to be around 1 in 370) and the phenotype does not generally include any signs or symptoms before cancer develops. For this reason (and due to the expense of testing for the broad range of mutations causing Lynch syndrome) it is not currently considered practical to screen for Lynch syndrome in the general population.

Instead, Lynch syndrome-causing mutations are sought amongst individuals more likely to have them, such as:

- Blood relatives of other individuals known to have Lynch syndrome (especially where a causative mutation is already documented);
- Individuals with a strong family history of colorectal and/or gynaecological cancer;
- Patients with colorectal cancer.

In this assessment the technologies are intended to be used for individuals in the last category, since they are performed using tumour cells and tumour DNA. MSI and MMR IHC test for evidence of lost MMR function. *MLH1* promoter hypermethylation testing and *BRAF* V600E testing are used to exclude MLH1-deficient tumours which are not caused by Lynch syndrome mutations. If a tumour is MMR-deficient then there is an increased probability that the individual has Lynch syndrome, and comprehensive mutation screening is offered.

If a constitutional Lynch syndrome-causing mutation is identified, predictive testing can then be offered to blood relatives. Predictive testing is generally less expensive than diagnostic testing.

The overall value proposition then, is that these technologies can be used to identify families with Lynch syndrome-causing mutations, and that surveillance and other risk-reducing interventions can be offered to them to reduce the risk of cancer (and therefore extend cancer-free and overall survival and reduce cancer-related costs) in a way that is a cost-effective use of NHS resources.

Objectives

To investigate whether testing for Lynch syndrome in colorectal cancer patients using MSI or IHC (with or without *MLH1* promoter hypermethylation testing and *BRAF* V600E testing) is clinically effective and whether it represents a cost-effective use of NHS resources.

This assessment comprises systematic reviews of published literature corresponding to:

- Diagnostic test accuracy studies of MSI and/or IHC in colorectal cancer patients;
- End-to-end studies of screening for Lynch syndrome in colorectal cancer patients;
- Economic evaluations of screening for Lynch syndrome in colorectal cancer patients.

In addition to these reviews, an independent economic evaluation is conducted using a simulation model.

Review of test accuracy

Methods

A systematic review was conducted to assess the diagnostic accuracy of molecular MSI testing and MMR immunohistochemistry (each with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing).

Bibliographic databases (including MEDLINE, Embase, Web of Science and The Cochrane Library) were searched using population terms for Lynch syndrome or hereditary non-polyposis colorectal cancer and intervention terms for MSI or IHC. Search results were limited by date from 2006 to current and to English language studies. In order to identify relevant studies published before 2006 (and any additional studies published after 2006) pre-specified systematic reviews were screened as well as any other systematic reviews identified by the bibliographic database searches.

Two reviewers independently assessed titles and abstracts returned by the search strategy, as well as full text papers, using pre-specified inclusion and exclusion criteria. These criteria stated that included studies should be single-gate or two-gate diagnostic studies (or a variation of these designs) recruiting individuals with colorectal cancer (CRC), and investigating the test accuracy of molecular MSI testing and/or MMR immunohistochemistry, with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing. The index test(s) must have been compared with a reference standard, and must provide data to enable at least the estimation of sensitivity. Other outcomes were: specificity, positive and negative likelihood ratios (LR+ and LR-), positive and negative predictive values (PPV and NPV), concordance with the reference standard, diagnostic yield, and test failure rates.

The reference standard was constitutional MMR mutation testing which, as a minimum, included DNA sequencing of *MLH1*, *MSH2* and *MSH6* and MLPA (or another appropriate technique for detecting large genomic abnormalities). The study must have been designed so that all participants would receive both the index test and reference standard, although for studies recruiting a representative sample of all CRC patients (including where an age limit was applied), it was acceptable for the reference standard to be applied to all index test positives and to a representative (e.g., random) sample of index test negatives. Studies published only as abstracts were only included if they were part of an included study that was also published in full. Disagreements between reviewers on whether a study should be included were resolved by discussion. To identify further studies, all full text includes were forward and backward citation chased.

For included studies, data extraction and quality appraisal (using Phase 3 of the QUADAS-2 tool) was performed by one reviewer (TJH) and checked by a second (HC). Any disagreements were resolved by discussion, with involvement of a third reviewer as necessary. For studies that were not based on high risk samples (including studies where the population was age-limited), sensitivity, specificity, LR+, LR−, PPV and NPV, with 95% CIs, were calculated, where data permitted. For studies based on high risk samples, only the sensitivity of the index test(s), with 95% CIs, was calculated. This is because the spectrum effects that occur when using high risk samples have previously not been found to lead to significant bias in sensitivity estimates (Palomaki, 2009). Studies that provided estimates of both sensitivity and specificity had their point estimates plotted in ROC space. Due to insufficient homogenous data, individual studies were not pooled in meta-analysis and results were discussed in a narrative synthesis.

Results

Summary of included studies

Ten studies met the test accuracy review inclusion criteria. One of the included studies (Poynter, 2008) had two distinct samples (a population-based sample and a high-risk sample); the results from all 11 samples are considered. Quality appraisal did not indicate a high risk of bias for any of the studies. Four of the included study samples are single-gate studies with population-based samples, including one apparently unselected CRC population (Poynter, 2008) and three age-limited populations (Barnetson, 2006; Limburg, 2011; Southey, 2005). Five of the study samples are single-gate studies based on high-risk populations (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005). The remaining two studies (Hendriks, 2003; Okkels, 2012) are a variation on a two-gate study design where participants with positive reference standard results were recruited but no participants with reference standard negative results were recruited. For this report, and for clarity, these studies have been termed “reference standard positive” studies.

Across the included studies there was variation in the reference standard including variation in: the sequencing methods and genes tested, techniques used to test for large genomic alterations and deletions, genes tested for large genomic alterations and deletions, whether unclassified variants were investigated. Test performance statistics were primarily generated with unclassified variants categorised as negative reference standard results. Only two studies provided data for secondary analyses where unclassified variants were categorised as positive reference standard results. None of the included studies made a direct comparison of MSI and IHC. As such, results are reported separately for these tests.

Summary of results for MSI

Nine samples provided data on MSI (Barnetson, 2006; Southey, 2005; both samples in Poynter, 2008; Caldes, 2004; Mueller, 2009; Overbeek, 2007; Shia, 2005; Hendriks, 2003). It should be noted that there was variation between studies in the MSI testing procedures used with regard to microdissection techniques, the panel of markers used, the way in which MSI was categorised (e.g., as a bimodal or trimodal categorisation), and the thresholds used to categorise MSI.

In primary analyses unclassified variants were categorised as negative reference standard results and MSI-L was considered to be a negative index test result. Studies that used a bimodal categorisation of MSI (i.e., MSI-positive or MSI-negative) were considered here and again where MSI-L was considered to be a positive index test result. Across all nine samples, when MSI-L was considered to be a negative index test result, sensitivity ranged from 66.7% (95% CI 47.2, 82.7) for the population-based sample reported by Barnetson et al. (2006) to 100.0% (95% CI 93.9, 100.0 for the population-based sample in Poynter, 2008; and 95% CI 85.8, 100.0 for the high-risk sample in Shia, 2005). Three population-based samples provided data to enable the calculation of specificity for MSI. Across these three samples, when MSI-L was considered to be a negative index test result, specificity ranged from 61.1% (95% CI 57.0, 65.1) in Poynter et al. (2008) to 92.5% (95% CI 89.1, 95.2) in Barnetson et al. (2006). It should be noted that Barnetson et al. (2006) was based on an age-limited sample whereas Poynter et al. (2008) was based on an unselected CRC population.

When MSI-L was considered to be a positive index test result sensitivity increased and specificity decreased. This was unsurprising; including MSI-L as a positive result essentially lowers the threshold for a positive index test result. The three population-based studies providing data on MSI were used to calculate likelihood ratios and predictive values; LR+, LR-, and PPV were decreased when MSI-L was considered to be a positive rather than a negative test result whereas on the whole the reverse was true for NPV.

Secondary analyses were also conducted, where data permitted, where unclassified variants were considered to be positive reference standard results. This was only possible for two studies (Caldes, 2004; Hendriks, 2003), and did not really alter results, probably owing to the low number of unclassified variants identified.

Summary of results for IHC

Seven samples (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks 2003) provided data to assess the accuracy of an overall IHC result at identifying a positive reference standard result, i.e., whether a positive IHC result, regardless of whether the result was for MLH1, MSH2, MSH6 (or for some studies, PMS2), identifies a positive reference standard result. Five study samples (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) split IHC data according to the particular protein assessed (MLH1, MSH2, MSH6 or PMS2), enabling an assessment of IHC for at least one of these individual proteins (i.e., whether an absence of a particular protein accurately identifies a mutation in that particular gene).

In primary analyses unclassified variants were categorised as negative reference standard results. Sensitivity estimates ranged from 80.8% (95% CI 60.6, 93.4) in Shia et al. (2005) to 100.0% (95% CI 81.5, 100.0) in Southey et al. (2005). The study by Southey et al. (2005) included an assessment of *PMS2* as well as MLH1, MSH2 and MSH6, and it is possible that this accounted for the higher sensitivity estimate. Nevertheless, all sensitivity estimates were >80%. Only two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary sensitivity estimates where unclassified variants were considered to indicate a positive reference standard test result. In both cases, sensitivity was reduced. Two population-based studies (Limburg, 2011; Southey, 2005) also provided data to enable the calculation of specificity. Specificity was estimated as 91.9% (95% CI 86.3, 95.7) for Limburg et al. (2011) and 80.5% (95% CI 65.1, 91.2) for Southey et al. (2005).

Three study samples provided data specific to the sensitivity of MLH1 loss of expression (Barnetson, 2006; Southey, 2005; Hendriks, 2003) with sensitivities ranging from 50.0% (95% CI 26.0, 74.0) for Southey et al. (2005) to 100.0% (95% CI 73.5, 100.0) for Barnetson et al. (2006). Three studies provided data specific to the sensitivity of MSH2 loss of expression with sensitivities ranging from 22.2% (95% CI 6.4, 47.6) for Southey et al. (2005) to 81.8% (95% CI 48.2, 97.7) for Barnetson et al. (2006). Four studies provided data specific to MSH6 loss of expression with sensitivities ranging from 44.4% (95% CI 21.5, 69.2) for Southey et al. (2005) to 75.0% (95% CI 19.4, 99.4) for Barnetson et al. (2006) and Hendriks et al. (2003). One study provided sufficient data to enable secondary sensitivity estimates, for individual proteins, where unclassified variants were considered to indicate a positive reference standard test result. These results were very similar to those estimated from data where unclassified variants were considered to be reference standard negatives. Specificities were also generated for the population-based studies. For MLH1 these ranged from 70.6% (95% CI 66.8, 74.2) for Poynter et al. (2008) to 96.0% (95% CI 93.1, 97.9) for

Barnetson et al. (2006); for MSH2 and MSH6 both studies provided a specificity >92%. Only the study by Southey et al. (2005) provided IHC data for PMS2, providing a sensitivity estimate of 55.6% (95% CI 30.8, 78.5) and a specificity estimate of 87.8% (95% CI 73.8, 95.9).

The population-based studies by Barnetson et al. (2006) and Southey et al. (2005) also provided data to enable the calculation of likelihood ratios and predictive values for individual proteins. For MLH1, MSH2 and MSH6, LR+ was greater in Barnetson et al. (2006) than in Southey et al. (2005) and for MSH2 and MSH6 LR− was lower in Barnetson et al. (2006) than in Southey et al. (2005) (only Southey, 2005 estimated LR− for MLH1). There are several possible reasons why these between-study differences occurred including the fact that Barnetson et al. (2006) was a larger study than Southey et al. (2005), that the reference standard was not identical in these studies, and that there is a possibility that IHC ratings may have differed across studies (interrater reliability). PPV and NPV for MLH1 and MSH2 were largely consistent across the two studies. For MSH6, NPV estimates were consistent across the two studies, but PPV estimates were vastly different, with data from Barnetson et al. (2006) resulting in a PPV of 16.7% (95% CI 3.6, 41.4) and data from Southey et al. (2005) resulting in a PPV of 72.7% (95% CI 39.0, 94.0). Although the reason for this difference is not completely clear, it is likely due, at least in part, to the very low number of true positive results (n=3) for loss of expression in MSH6 in the study by Barnetson et al. (2006).

Review of end-to-end studies

Methods

A literature search was conducted to identify end-to-end studies of screening for Lynch syndrome in colorectal cancer patients, i.e., studies which compare patients receiving screening to patients not receiving screening in terms of long-term outcomes, such as survival and cancer incidence.

The same search strategy was employed as for test accuracy reviews (i.e., no study design filters were employed). One experienced researcher screened all titles and abstracts for eligibility and a second researcher checked a 10% random sample.

Results

No eligible studies were identified. Some pre-post studies (i.e., studies which compared outcomes before and after the introduction of screening) were identified which had only been published in abstract and could not be included.

Review of cost-effectiveness evidence

Methods

The following databases were searched for economic studies: MEDLINE (Ovid), MEDLINE In-Process & Other Non-Indexed Citations (Ovid), Embase (Ovid), Web of Science (Thomson Reuters), NHS EED (The Cochrane Library), EconLit (EBSCO). All searches were limited to English language studies where possible, and a date limit of 2013 was used to reflect that this was an update of the systematic review of cost-effectiveness reported in

Snowsill et al. (2014). Reviews by Snowsill et al. (2014) and Grosse (2015) were also considered as additional sources.

After two reviewers completed the screening process, the bibliographies of included papers were scrutinised for further potentially includable studies. The inclusion criteria were as follows:

- **Population** All people with newly diagnosed colorectal cancer
- **Intervention** Microsatellite instability testing (with or without BRAF V600E mutation testing and with or without *MLH1* methylation testing), or immunohistochemistry (with or without BRAF V600E mutation testing and with or without *MLH1* methylation testing)
- **Comparator** The included interventions, no testing, or direct constitutional MMR gene mutation testing
- **Outcomes** Costs and health effects measured in life years or QALYs
- **Study type** Decision analytic models, economic evaluations within trials, or cost or resource use studies from the UK

Studies were critiqued using summary tables and narrative synthesis and full papers were quality appraised using selected criteria from the Drummond checklist.

Results

Of 352 search results, 6 publications were identified and reviewed. Of the 33 additional publications identified in the previous reviews, 4 were identified and reviewed. Two publications reported the same study, giving a total of 9 included studies.

Seven studies were US based, 1 German and 1 study was UK based. All studies included strategies to identify Lynch syndrome in people with CRC and their relatives. Modelling was similar across studies, with a diagnostic model to identify Lynch syndrome and a long term model to estimate the costs and benefits associated with the outcomes of the diagnostic model. Five studies were cost-utility studies.

The studies identified reported a wide variety of analyses, with varying quality in reporting. No single study answered our decision problem in full and the most common reason for this was that they did not include all the interventions identified by the NICE Scope or they were not from a UK perspective and therefore hard to generalise.

Most studies stated that at least one strategy to identify Lynch syndrome could be cost-effective according to their perspective and when a universal genetic testing strategy was present, strategies that used tumour based tests to enrich the population appeared to improve cost-effectiveness (reducing ICERs). Most models agreed that effectiveness of colonoscopy screening, number of relative and prevalence of Lynch syndrome impacted the cost-effectiveness of the models the most.

The economic analysis most relevant to the decision problem was Snowsill et al. (2014), which was a UK-based, cost-utility analysis, and well-reported. It was decided that this model could be adapted and developed to suit the current decision problem.

Independent economic evaluation

Methods

A previously developed model was adapted and extended for the current decision problem. The model comprises two components: a decision tree to model diagnostic outcomes from screening for Lynch syndrome, and an individual patient Monte Carlo simulation to model long-term outcomes. The decision tree component is deterministic while the simulation model is stochastic.

Diagnostic model

The diagnostic component models ten different strategies to test for Lynch syndrome in colorectal cancer patients:

1. No reflex testing for Lynch syndrome;
2. IHC, followed by comprehensive MMR mutation testing if IHC abnormal result;
3. IHC, followed by:
    - If MLH1 abnormal: *BRAF* testing, followed by comprehensive MMR mutation testing if *BRAF* wild type;
    - If IHC abnormal except MLH1: comprehensive MMR mutation testing;
4. IHC, followed by:
    - If MLH1 abnormal: *MLH1* methylation testing, followed by comprehensive MMR mutation testing if *MLH1* not methylated;
    - If IHC abnormal except MLH1: comprehensive MMR mutation testing;
5. IHC, followed by:
    - If MLH1 abnormal: *BRAF* testing, followed by *MLH1* methylation testing if *BRAF* wild type, followed by comprehensive MMR mutation testing if *MLH1* not methylated;
    - If IHC abnormal except MLH1: comprehensive MMR mutation testing;
6. MSI, followed by comprehensive MMR mutation testing for MSI result;
7. MSI, followed by *BRAF* testing for MSI result, followed by comprehensive MMR mutation testing if *BRAF* wild type;
8. MSI, followed by *MLH1* methylation testing for MSI result, followed by comprehensive MMR mutation testing if *MLH1* not methylated;
9. MSI, followed by *BRAF* testing, followed by *MLH1* methylation testing if *BRAF* wild type, followed by comprehensive MMR mutation testing if *MLH1* not methylated;
10. Comprehensive MMR mutation testing.

Where a colorectal cancer patient (a *proband*) is diagnosed with Lynch syndrome and a specific pathogenic MMR mutation is identified, cascade predictive genetic testing is offered to relatives. Where a proband is diagnosed with Lynch syndrome but no specific mutation is identified, the first-degree relatives of the proband are offered colonoscopic surveillance.

The sensitivity and specificity of different tests were drawn from the literature, and from a meta-analysis of population-based test accuracy studies identified in the review of test accuracy studies for MSI and IHC. These were combined with estimates of the prevalence of Lynch syndrome in colorectal cancer patients and other parameters to estimate the number of probands and relatives who would be correctly diagnosed with Lynch syndrome (true positives; TP), incorrectly diagnosed with Lynch syndrome (false positives; FP), incorrectly not diagnosed with Lynch syndrome (false negatives; FN) and correctly not diagnosed with Lynch syndrome (true negatives; TN).

The costs of diagnostic tests were estimated from directly reported UK costs from molecular genetics laboratories and pathology departments. The psychological impact of a positive genetic diagnosis or of declining genetic testing on health-related quality of life was estimated based on a US vignette study.

Outcomes model

An event-driven individual patient simulation was used to estimate long-term outcomes for individuals according to their starting characteristics:

- Proband or relative;
- Male or female;
- Truly has Lynch syndrome-causing mutation;
- Diagnosed with Lynch syndrome;
- Accepted Lynch syndrome surveillance colonoscopies (if diagnosed with Lynch syndrome).

These characteristics jointly define 24 groups, and for each of these groups 10,000 individual patients were simulated.

The events simulated were:

- Colorectal cancer incidence (Stage I to Stage IV);
- Metachronous colorectal cancer incidence (for individuals with previous colorectal cancer; Stage I to Stage IV);
- Colorectal cancer mortality;
- Colonoscopy;
- Non-fatal colonoscopy complication;
- Fatal colonoscopy complication;
- Endometrial cancer incidence;
- Endometrial cancer mortality;
- Enter/exit gynaecological surveillance;
- Prophylactic hysterectomy and bilateral salpingo-oophorectomy (H-BSO);
- Mortality from prophylactic H-BSO;
- General mortality (from other causes).

The risks of cancer incidence and mortality were estimated from the published literature or from national statistics (generally for England). The uptake of gynaecological surveillance and prophylactic H-BSO was estimated from an audit of the Northern Genetics Service.

The costs of cancer treatment were estimated based on published literature. The costs of colonoscopy, colonoscopy complications, gynaecological surveillance and prophylactic H-BSO were estimated from NHS reference costs.

The health-related quality of life for individuals was modelled using a baseline utility (which was age and sex dependent) and utility decrements estimated from the published literature for cancer.

Colonoscopic surveillance was assumed to reduce the risk of colorectal cancer incidence, and to affect the stage distribution of colorectal cancers (which in turn reduces average mortality), as estimated from the published literature. Gynaecological surveillance was assumed to reduce the mortality from endometrial cancer but not incidence based on a previously conducted review of the literature which found evidence suggesting an improved stage distribution. Prophylactic H-BSO was assumed to completely eliminate the risk of endometrial cancer. Aspirin was assumed to reduce the incidence of colorectal cancer and endometrial cancer.

Summary of assumptions

The following is a list of assumptions, with key assumptions highlighted in bold:

- Diagnosis:
  - **Assumed to occur without delay;**
  - Assume no surveillance prior to diagnosis;
  - Predictive testing (in relatives) assumed to be 100% accurate;
  - MSI-L treated as negative test result;
  - **Sensitivity of MSI and IHC assumed independent of MMR gene mutated;**
  - **Sensitivity of diagnostic mutation testing (in probands) conservatively assumed to be 90% (MLH1, MSH2 and MSH6) or 62% (PMS2);**
  - Test accuracy assumed to be independent of patient characteristics, **including age**, sex and tumour location (colon versus rectum);
  - **No follow-up testing, e.g., testing for other hereditary colorectal cancer syndromes if Lynch syndrome is not diagnosed;**
  - **All four Lynch syndrome genes tested for mutations unless additional tumour tests (BRAF and/or MLH1 methylation testing) also conducted, in which case only MLH1 and PMS2 tested;**
- Cascade testing:
  - **On average, six relatives (2.5 of these first-degree relatives) are offered genetic counselling per proband with Lynch syndrome;**
  - 44% chance each relative tested has Lynch syndrome;
- Colorectal cancer:

    - Synchronous colorectal tumours not modelled;
    - Mortality risks from cancers are additive in the hazard rate;
    - Colorectal cancer stage assumed to be dependent only on whether individual is undergoing Lynch syndrome colonoscopic surveillance;
- Endometrial cancer:
    - Not modelled for women without Lynch syndrome-causing mutations;
    - Treatment assumed to be H-BSO ± chemotherapy ± radiotherapy;
    - **Survival of endometrial cancer not affected by Lynch syndrome status;**
- Surveillance:
    - **Surveillance colonoscopies reduce colorectal cancer incidence in unaffected individuals by 61% (hazard ratio 0.387) and reduce metachronous colorectal cancer incidence by 47% (hazard ratio 0.533);**
    - **Surveillance colonoscopies improve the stage distribution of colorectal cancer from 44.6% early (Stage I/II) to 79.1% early;**
    - **Biennial colonoscopies (2-year intervals) for colorectal surveillance, starting at age 25 years and stopping at age 75 years;**
    - **Surveillance colonoscopies are effective immediately upon commencement of surveillance and ineffective immediately after discontinuation (i.e., no lag time);**
    - **No crossover into or out of colorectal surveillance;**
    - Risk of adverse events is independent of patient characteristics (e.g., age);
    - Annual gynaecological examination, transvaginal ultrasound, endometrial biopsy and CA-125 testing for gynaecological surveillance;
    - Gynaecological surveillance reduces endometrial cancer mortality by 10% (hazard ratio 0.898);
- Prophylactic H-BSO:
    - No disutility in base case;
    - Assumed to eliminate risk of endometrial cancer;
- Aspirin:
    - 80.4% of patients offered aspirin;
    - 59% of those patients offered aspirin receive it for four years, the remainder receive none;
    - **Patients receiving aspirin for four years have a reduction in colorectal cancer incidence (hazard ratio 0.37) and endometrial cancer incidence (hazard ratio 0.49);**
    - **Hazard ratio applies immediately and lasts for ten years;**

    - No adverse events from aspirin;
    - 600 mg daily dose;
- Costs:
    - Unit costs are independent of patient characteristics, e.g., age, sex;
    - **Lifetime (undiscounted) costs of colorectal cancer are around £47,000;**
    - Lifetime (undiscounted) costs of endometrial cancer are around £7,000;
    - Gynaecological surveillance costs £473 per year;
- Utilities:
    - **Disutility from colorectal cancer is dependent only on cancer stage and does not vary with time since diagnosis (i.e., lasts until death);**
    - **In base case disutility applies only to Stage IV (metastatic) colorectal cancer (0.13);**
    - Disutility from endometrial cancer (0.036) applies for one year only;
- Other:
    - **Ovarian cancer, small bowel cancer, gastric cancer and other Lynch syndrome-associated cancers not modelled.**

Results

Base case results

In the base case analysis, four strategies were on the cost-effectiveness frontier, i.e., they were not dominated (more expensive and less effective than another strategy) or extended dominated (more expensive and less effective than some combination of other strategies). These were (in ascending cost order and presented with fully incremental ICERs):

- Strategy 1 (No testing): Referent strategy
- Strategy 5 (IHC → *BRAF* → *MLH1* methylation → Genetic testing): £11,008/QALY
- Strategy 3 (IHC → *BRAF* → Genetic testing): £37,495/QALY
- Strategy 2 (IHC → Genetic testing): £60,967/QALY

At a cost-effectiveness threshold of £20,000 to £30,000 per QALY, Strategy 5 is therefore predicted to be cost-effective. For each annual cohort, Strategy 5 is predicted to provide an incremental net health benefit (versus Strategy 1, at a willingness-to-pay of £20,000 per QALY) of 847.5 QALYs, which results from incremental costs of £20.75 million and 1,885 additional QALYs (over the lifetime of the cohort, outcomes discounted at 3.5% per annum).

Life expectancy for individuals with Lynch syndrome is improved by 1.2 years for probands and 2.1 years for relatives (Strategy 5 versus Strategy 1). Over 300 colorectal cancers are expected to be prevented over the lifetime of each annual cohort.

Subgroup analyses

Subgroup analyses were conducted according to the age of the probands (<50, <60, <70, >70). In all subgroup analyses Strategy 5 was on the cost-effectiveness frontier with an ICER below £20,000 per QALY. When an age limit of 50 years is imposed for probands, Strategy 3 is marginally cost-effective with an ICER of £19,903 per QALY.

Scenario analyses

In all these scenario analyses the strategies on the cost-effectiveness frontier were unchanged from the base case.

When MSI-L results were treated as positive and led to further testing, the cost-effectiveness for these strategies worsened, but these strategies were not on the cost-effectiveness frontier in the base case.

When aspirin was removed from the model, costs increased slightly and clinical outcomes worsened slightly, resulting in an overall worsening of cost-effectiveness for testing strategies. The ICER for Strategy 5 increased to £11,659 per QALY.

When gynaecological surveillance was assumed to take place but have no clinical benefit, clinical outcomes worsened. The ICER for Strategy 5 was £11,375 per QALY.

When gynaecological surveillance was removed from the model, the ICER for Strategy 5 was £10,241 per QALY.

When the disutilities for colorectal cancer were estimated from an alternative source, the ICER for Strategy 5 was £9,775 per QALY.

When colonoscopic surveillance was assumed not to reduce the incidence of colorectal cancer (worst case scenario), a number of strategies were no longer cost-effective versus Strategy 1 (no testing). The ICER for Strategy 5 was £19,194 per QALY.

Sensitivity analyses

A number of deterministic sensitivity analyses were conducted. Here, only the sensitivity analyses resulting in worsened cost-effectiveness are reported.

The diagnostic accuracy of tests had an impact on cost-effectiveness. When the sensitivity and specificities of all tumour tests are reduced to their lower 95% confidence limits the ICER for Strategy 5 increased to £16,036 per QALY and MSI-based strategies were no longer predicted to be cost-effective versus Strategy 1 at a threshold of £20,000 per QALY.

When the probabilities of colorectal cancer patients accepting genetic counselling and genetic testing are reduced to 50% (from values ≥90% in the base case) the ICER for Strategy 5 increased to £17,767 per QALY.

When no relatives were assumed to be identified for cascade testing, the ICER for Strategy 5 increased to £17,921 per QALY.

The lifetime colorectal cancer risk for individuals with Lynch syndrome-causing mutations has a strong impact on cost-effectiveness. At the lower 95% confidence limit, the ICER for Strategy 5 increased to £19,300 per QALY.

Discussion

The systematic reviews were conducted according to a pre-specified protocol and with searches designed and conducted by an information specialist. The searching strategy did not include a search for grey literature and the searches were conducted in February 2016.

The economic evaluation was based on a previously developed economic model which had been quality assured and peer reviewed. The complexity of the modelling allowed for very many of the aspects of the diagnosis and management of Lynch syndrome to be incorporated in the assessment of cost-effectiveness, but also meant that a PSA could not be conducted. Ovarian cancer and other less common Lynch syndrome-associated cancers were not modelled.

There is some concern about the generalisability of the results of the diagnostic test accuracy systematic review, given most of the studies were in high-risk or age-limited populations, rather than the unselected colorectal cancer population which is specified in the decision problem.

Conclusions

Findings from the review of test accuracy studies suggest that MSI and IHC are effective but imperfect tests to identify colorectal cancer patients who may have Lynch syndrome. Due to the limited number of studies identified, the known differences in their methods, and the observed heterogeneity in their results, it is thought inappropriate to produce a single point estimate for the diagnostic accuracy measures for these tests, but both tests are capable of enriching a population for genetic testing.

There is no high-quality evidence from end-to-end studies that screening for Lynch syndrome in colorectal cancer patients improves long-term outcomes such as survival or cancer incidence, and such evidence is unlikely to be produced in the future.

Previous economic evaluations have suggested that it may be cost-effective to screen for Lynch syndrome in colorectal cancer patients using clinical criteria, decision tools and/or tumour-based tests.

The current economic evaluation, which directly addresses the decision problem, suggests that screening for Lynch syndrome is cost-effective at a cost-effectiveness threshold of £20,000 to £30,000 per QALY.

Scenario and sensitivity analyses revealed that two parameters which cannot be easily estimated have a significant impact on cost-effectiveness: the effectiveness of surveillance colonoscopy in reducing CRC incidence, and the lifetime risk of colorectal cancer for individuals with Lynch syndrome-causing mutations. The estimates used for modelling are believed to be the most suitable from the scientific literature, considering the possible sources of bias and the size of the relevant studies.

Recommendations for research

We recommend research into the effectiveness and cost-effectiveness of screening for Lynch syndrome in endometrial cancer patients (and considering age-based subgroups as in this analysis).

We also note that some have suggested utilising next-generation sequencing panels which can identify certain mutations across a wide range of cancer predisposition genes on all

colorectal cancer patients (or early-onset patients, or those with a family history of colorectal cancer). We recommend research into the potential effectiveness and cost-effectiveness of such strategies compared to the strategies analysed here.

Plain English Summary

The DNA mismatch repair system corrects errors in DNA replication, which occurs when cells in the body divide. Without the mismatch repair system, errors in DNA multiply and this can lead to cancer developing, especially in the bowel and female reproductive organs.

Most people are born with two working copies of the genes responsible for the mismatch repair system, but some people inherit a faulty (mutated) copy for one of the genes. Since they have only one working copy they are more likely to lose the function in their mismatch repair system, and then get cancer. This leads to patterns of cancer in a family, which is known as Lynch syndrome.

The aim of this study was to find out whether it would be clinically effective (i.e., good for patients and their families) and cost-effective (i.e., a good use of limited NHS resources) to screen bowel cancer patients for Lynch syndrome using tests on their tumours. If tests on the tumours suggest the patient has Lynch syndrome they can be offered genetic testing to search for the mutated gene responsible. Family members can then be offered a blood test to see if they also have the mutated gene. The benefit of knowing someone has a mutation which causes Lynch syndrome is that surveillance can be offered, such as colonoscopy, to reduce the risk of cancer in the future.

Although it has not been proven in practice, a mathematical model suggests that screening would be clinically effective and cost-effective.

Word count: 248 words

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Abbreviations

5-FU	fluorouracil
aGCH	array-based comparative genomic hybridization
ACPGBI	Association of Coloproctology of Great Britain and Ireland
AJCC	American Joint Committee on Cancer
AMS II	Amsterdam II criteria
APER	abdominoperineal excision of the rectum
AR	anterior resection
AUC	area under the curve
BRAF	a human gene that makes a protein called B-raf (a member of the raf kinase family)
BRAF V600E	a mutation of the *BRAF* gene detected in a range of carcinomas, including CRC
BSG	British Society of Gastroenterologists
CA125	cancer antigen 125 (a protein biomarker for certain cancers)
CAPP2	Colorectal Adenoma/Carcinoma Prevention Programme 2
CaPP3	Cancer Prevention Programme 3
C(A)T	computed axial tomography
CC	clinical criteria
CCT	Controlled clinical trial
CEA	carcinoembryonic antigens test
CEA	cost-effectiveness analysis
CI	confidence interval
CpG	cytosine—phosphate—guanine (sequence of DNA bases)
CRC	colorectal cancer

CRD	NHS Centre for Reviews and Dissemination
CSGE	Conformation-sensitive gel electrophoresis
DGGE	denaturing gradient gel electrophoresis
DHPLC	denaturing high performance liquid chromatography
DNA	deoxyribonucleic acid
DTA	diagnostic test accuracy
EBRT	external beam radiation therapy
E(n)C(a)	endometrial cancer
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EORTC	European Organisation for Research and Treatment of Cancer
EPCAM	Epithelial cell adhesion molecule
EQ-5D(-3L)	EuroQol Five Dimensions (three levels); a generic health-related quality of life questionnaire
FACT-G	Functional Assessment of Cancer Therapy – General; a health-related quality of life questionnaire for cancer therapy
FAP	Familial Adenomatous Polyposis
FDR	first degree relative
FIGO	International Federation of Gynaecology and Obstetrics
FH	family history
FN	false negative
FOI	Freedom of Information
FP	false positive
GC	genetic counselling
GFR	glomerular filtration rate
GHS	global health status
GP	general practitioner
GP	general population

GT	genetic testing
H-BSO	hysterectomy and bilateral salpingo-oophorectomy
HCHS	Hospital and Community Health Services
HNPCC	Hereditary non-polyposis colorectal cancer
HR	hazard ratio
HRG	Healthcare Resource Group
HRQ(o)L	health related quality of life
HTA	Health Technology Assessment
ICD	International Classification of Diseases
ICER	incremental cost effectiveness ratio
IHC	immunohistochemistry
(I)N(H)B	(incremental) net (health) benefit
InSiGHT	International Society for Gastrointestinal Hereditary Tumours
IPAA	ileal pouch anal anastomosis
LR+	likelihood ratio for positive test result
LR-	likelihood ratio for negative test result
LS	Lynch syndrome
LYG	life year gained
mCRC	metachronous CRC
MMR	mismatch repair
MLH1	MutL homolog 1
MLPA	multiplex ligation-dependant probe amplification
mRNA	messenger ribonucleic acid
MSI	microsatellite instability
MSI-H	MSI-high
MSH2	MutS homolog 2

MSH6	MutS homolog 6
MSI-L	MSI-low
MSS	Microsatellite stable
MTA	multiple technology assessment
N/A	not applicable
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NCIN	National Cancer Intelligence Network
NE	Not estimated/estimable
NGS	Next generation sequencing
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence
NIH	National Institutes of Health
NIHR	National Institute for Health Research
NPV	negative predictive value
NR	not reported
ONS	Office for National Statistics
OS	overall survival
PCR	polymerase chain reaction
PD-1	Programmed cell death protein 1
PenTAG	Peninsula Technology Assessment Group
PMS2	postmeiotic segregation increased 2
PORTEC	Postoperative Radiation Therapy for Endometrial Carcinoma
PPV	positive predictive value
PSA	probabilistic sensitivity analysis
PSS	personal social services

PSSRU	Personal Social Services Research Unit
QALY	quality-adjusted life year
QLQ-C30	a health-related quality of life questionnaire for cancer patients
QLQ-CR29	an add-on module to the QLQ-C30 for colorectal cancer patients
QoL	quality of life
QUADAS-2	tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews
RBG	Revised Bethesda Guidelines
RCPath	Royal College of Pathologists
RCT	randomised controlled trial
ROC	receiver operating characteristic
SA	sensitivity analysis
SDR	second degree relative
SEG	segmental colon resection
SF-12	Short form 12 questions; a general health-related quality of life tool
SF-36	Short form 36 questions; a general health-related quality of life tool
SF-6D	Short form 6 domains; a general health-related quality of life tool
SROC	summary receiver operating characteristic
SSCP	single-strand conformational polymorphism
SUB	subtotal colectomy
TAHSBO	Total abdominal hysterectomy with bilateral salpingo-oophorectomy
TBT	tumour based test
TN	true negative
TNM	tumour-node-metastasis
TP	true positive
TTO	time trade-off

UK	United Kingdom
UKAS	United Kingdom Accreditation Service
UKGTN	UK Genetics Testing Network
UV	unclassified variants
VBT	vaginal brachytherapy
VUS	variant of unknown/uncertain significance

Glossary

Adenoma	A benign tumour formed from glandular structures in epithelial tissue
Adjuvant chemotherapy	Additional cancer treatment given after the primary treatment to lower the risk that the cancer will come back
Amsterdam criteria	A set of diagnostic criteria used by doctors to help identify families which are likely to have Lynch syndrome
Anastomosis	A connection made surgically between adjacent blood vessels, parts of the intestine, or other channels of the body
Anterior resection	Removal of an area of the rectum and/or left side of the bowel
Array-based comparative genomic hybridization	A cytogenetic technology that evaluates areas of the human genome for gains or losses of chromosome segments
Base case	The expected case using the assumptions deemed most likely to occur
Bethesda panel of MSI markers	A panel of 5 microsatellite markers proposed in the original Bethesda guidelines to describe microsatellite instability (MSI) in Lynch syndrome (BAT25, BAT26, D2S123, D5S346, D17S250)
Bimodal distribution of MSI	Tumours defined as positive for negative for MSI
Ca125 analysis	Blood test for ovarian cancer
Carcinoembryonic antigen (CEA) test	Carcinoembryonic antigens are harmful substances (usually proteins) that are produced by some types of cancer. In response to the antigens, the body produces antibodies to help fight them. A CEA test is often carried out after surgery to check carcinoembryonic antigen levels.
Carcinogenesis	The formation of a cancer, whereby normal cells are transformed into cancer cells
Cascade testing	The identification of close relatives of an individual with a disorder to determine whether the relatives are also affected or are carriers of the same disorder

Chemoprevention	The use of pharmacologic or natural agents that inhibit the development of invasive cancer either by blocking the DNA damage that initiates carcinogenesis or by arresting or reversing the progression of premalignant cells in which such damage has already occurred
Colonoscopic surveillance	A screening programme in people at high risk of developing colorectal cancer in order to detect precancerous changes early on and potentially prevent progression
Concordance	The degree of agreement between two diagnostic tests
Conformation-sensitive gel electrophoresis	A method for rapid detection of single-base differences in double-stranded PCR products and DNA fragments
Confounder	A third variable that can make it appear (sometimes incorrectly) that an observed exposure is associated with an outcome
Constitutional genetic testing	Tests for mutations that affect all cells in the body and have been there since conception (also known as germline testing)
Constitutional mutation	A genetic mutation present in all cells (also known as germline)
Cost-effectiveness analysis	An economic analysis that converts effects into health terms and describes the costs for additional health gain.
Cytotoxicity	The quality of being toxic to cells
Decision modelling	A theoretical construct that allows the comparison of the relationship between costs and outcomes of alternative healthcare interventions
Decision tree	A decision support tool that uses a tree-like graph or model of decisions and their possible consequences, including chance event outcomes, resource costs, and utility
Deletion	Change in the number of DNA bases by removing a piece of DNA
Denaturing gradient gel electrophoresis	A molecular fingerprinting method that separates polymerase chain reaction (PCR)-generated DNA products
Denaturing high performance liquid chromatography	A method of chromatography for the detection of base substitutions, small deletions or insertions at the DNA

Diagnostic yield	The number of positive test results divided by the number of samples
Dinucleotide	A nucleotide consisting of two units each composed of a phosphate, a pentose, and a purine or pyrimidine base
Discounted costs	A method of valuation using the concepts of the time value of money. Future costs are estimated and discounted by using cost of capital to give their present value.
Disutility	The adverse or harmful effects associated with a particular activity or process, especially when carried out over a long period
DNA mismatch repair (MMR)	A process that corrects mismatches generated during DNA replication
Dukes' stage	The Dukes' cancer staging system is divided into 4 groups - A, B, C and D. Dukes' A is an early bowel cancer and Dukes' D is advanced.
Duplication	Consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein
Dysplasia	The enlargement of an organ or tissue by the proliferation of cells of an abnormal type, as a developmental disorder or an early stage in the development of cancer
End-to-end study	Studies that follow patients from testing, through treatment, to final outcomes
Exteriorisation	To transpose an internal organ to the exterior of the body
False negative	Incorrect negative test result – number of diseased persons with a negative test result
False positive	Incorrect positive test result – number of non-diseased persons with a positive test result
First degree relative	A person's parent, sibling, or child
Frameshift mutation	Occurs when the addition or loss of DNA bases changes a gene's reading frame. The resulting protein is usually non-functional
Germline	Inherited material that comes from the eggs or sperm and is passed on to offspring

Germline mutation	A detectable and heritable variation in the lineage of germ cells, which is subsequently transferred to offspring and gives rise to constitutional mutation
Hazard ratio	A measure of how often a particular event happens in one group compared to how often it happens in another group, over time
Hereditary non polyposis colorectal cancer	Previous name for Lynch syndrome. A hereditary disorder that causes an increase in the risk of several types of cancer.
Hypermethylation	An increase in the epigenetic methylation of cytosine and adenosine residues in DNA
Hysterectomy	A surgical procedure to remove the womb (uterus)
Immunoreactivity	A measure of the immune reaction caused by an antigen
Incidence	The rate of new (or newly diagnosed) cases of a disease
Incremental cost effectiveness ratio	The difference in the mean costs of two interventions in the population of interest divided by the difference in the mean outcomes in the population of interest
Index test	The test whose performance is being evaluated
Intention-to-treat analysis	Includes every subject who is randomised according to randomised treatment assignment. It ignores noncompliance, protocol deviations, withdrawal, and anything that happens after randomisation.
Insertion	Changes the number of DNA bases in a gene by adding a piece of DNA. As a result, the protein made by the gene may not function properly.
Interrater variability	The degree of agreement among raters (histopathologists for IHC)
Meta-analysis	Statistical techniques used to combine the results of two or more studies and obtain a combined estimate of effect
Likelihood ratio	The likelihood that a given test result would be expected in a patient with the target disorder compared to the likelihood that that same result would be expected in a patient without the target disorder
Loco-regional metastases	Metastasis (spread) of a cancer only within the region in which it arose

Markov model	A stochastic model describing a sequence of possible events in which the probability of each event depends only on the state attained in the previous event
Metastatic disease	The spread of cancer from one organ or body part to another organ or body part
Metachronous tumours	Primary tumours not occurring at the same time (usually occurring more than 6 months apart)
Methylation	A process by which methyl groups are added to DNA.
Microdissection	Refers to a variety of techniques where a microscope is used to assist in dissection
Microsatellite instability (MSI)	Abnormal patterns of microsatellite repeats observed when DNA is amplified from a tumour with defective MMR compared with DNA amplified from surrounding normal tissue
Microsatellite stable (MSS)	No evidence of abnormal patterns of microsatellite repeats or defective MMR
Mismatch repair genes	Genes are involved in repairing DNA synthesis errors, repairing double-strand DNA breaks, apoptosis, antirecombination and, destabilization of DNA.
Missense	A change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene
Mixed-effects logistic regression	Used to model binary outcome variables, in which the log odds of the outcomes are modelled as a linear combination of the predictor variables when data are clustered or there are both fixed and random effects
Mononucleotide	A nucleotide that is derived from one molecule each of a nitrogenous base, a sugar, and a phosphoric acid
Mortality bias	Where mutation carriers are more likely to have died before being able to receive predictive testing
Multiplex ligation-dependent probe amplification	A multiplex PCR method detecting abnormal copy numbers of up to 50 different genomic DNA or RNA sequences, which is able to distinguish sequences differing in only one nucleotide
Negative predictive value	This is the probability of someone with a negative test result actually not having the disease
Net survival	The survival calculated from the estimated excess hazard of mortality caused by a condition

Next generation sequencing	Non-Sanger-based high-throughput DNA sequencing technologies
Nonsense	A change in one DNA base pair that results in a premature signal to stop building a protein. This type of mutation results in a shortened protein that may function improperly or not at all.
Optimum cut off	The cut off score which demonstrates the best trade-off between sensitivity and specificity
Pathogenic mutation	A mutation capable of causing disease
Penetrance	The proportion of individuals carrying a particular variant of a gene (allele or genotype) that also expresses an associated trait
Per-protocol analysis	A comparison of treatment groups that includes only those patients who completed the treatment originally allocated
Polymerase chain reaction (PCR)	A technology used for amplifying DNA sequences
Positive predictive value	The probability of someone with a positive result actually having the disease
Predictive testing	Testing for known mutations
Prevalence	The proportion of cases in the population at a given time
Primary tumour	A tumour growing at the anatomical site where tumour progression began
Probabilistic sensitivity analysis	A technique to quantify the level of confidence that a decision-maker has in the conclusions of an economic evaluation.
Proband	The first affected family member
Promoter	A region of DNA that initiates transcription of a particular gene
Proctocolectomy	Surgical removal of the rectum and all or part of the colon
Proximal colon	The first and middle parts of the colon
Quality adjusted life year (QALY)	A measure of health gain, used in economic evaluations, in which survival duration is weighted or adjusted by the patient's quality of life during the survival period.
Receiver Operating Characteristic (ROC) curve	A graph which illustrates the trade-offs between sensitivity and specificity which result from varying the diagnostic threshold.

Reference costs	The average unit cost to the NHS of providing secondary healthcare to NHS patients
Reference standard	The best currently available diagnostic test, against which the index test is compared
Reference standard positive study	Studies that used a variation on a two-gate study design where only participants who were reference standard positives were recruited
Reflex testing	Testing performed automatically in response to patient characteristics or the results of other tests
Regional metastases	The spread of cancer beyond the initial site to regional lymph nodes.
Relative survival	The observed survival within a group (e.g., people with colorectal cancer) as a proportion of the expected survival for a group with the same age and sex distribution
Revised Bethesda Guidelines	Recommendations for identifying individuals with Lynch syndrome
Salpingo-oophorectomy	Surgical removal of a fallopian tube and an ovary
Scenario analysis	A process of analysing possible future costs by considering alternative possible outcomes
Second degree relative	Someone who shares 25% of a person's genes. It includes uncles, aunts, nephews, nieces, grandparents, grandchildren, and half-siblings.
Segmental resection	Surgeons remove the cancer, a section of normal colon on either side of the cancer, and nearby lymph nodes, and then reattach the sections of the remaining colon
Sensitivity	Proportion of individuals with the target disorder who have a positive test result.
Sensitivity analysis	A technique used to determine how different values of an independent variable impact a particular dependent variable under a given set of assumptions
Single gate study	Where a single sample of individuals is assessed by both the index test and reference standard

Single-strand conformational polymorphism	A conformational difference of single-stranded nucleotide sequences of identical length as induced by differences in the sequences under certain experimental conditions. This allows sequences to be distinguished by means of gel electrophoresis.
Somatic	Referring to the cells of the body in contrast to the germ line cells
Southern blot analysis	A procedure for identifying specific sequences of DNA, in which fragments separated on a gel are transferred directly to a second medium on which assay by hybridization may be carried out
Specificity	Proportion of individuals without the target disorder who have a negative test result
Spectrum bias	The phenomenon that the performance of a diagnostic test may vary in different clinical settings because each setting has a different mix of patients
Splenic flexure	A curvature on the left of the transverse colon
Splice site	Causes abnormal mRNA processing
Sporadic CRC	CRC with no apparent hereditary component
Subtotal colectomy	An operation to remove the colon, leaving the rectum behind
Synchronous colorectal tumours	Primary tumours diagnosed within 6 months of each other
Trimodal distribution of MSI	Threshold distribution which includes MSI-H, MSI-L and MSS
Tumorigenesis	The production or formation of a tumour or tumours
Two gate study	Studies which employ separate sampling schemes for diseased and non-diseased participants, with both groups being assessed by the index test
Unclassified variant	A variation in a genetic sequence whose association with disease risk is unknown. Also called variant of uncertain significance, variant of unknown significance, and VUS.
Univariate analysis	The examination of one variable at a time
Variants of uncertain significance	A variation in a genetic sequence whose association with disease risk is unknown. Also called unclassified variant.

1 Background and definition of the decision problem(s)

Colorectal cancer (CRC) is the second most common cause of cancer deaths in the UK (2012: 16,187 deaths, 10% of all cancers).[1] It is considered to be a multifactorial disease, with environment and inheritance playing varying roles in different patients.[2] The majority of individuals with CRC have sporadic disease (approximately 70 to 80%). However, the remaining 20 to 30% have a family history of the disease, with 5% to 6% of these diagnosed with mutations of a known hereditary cancer syndrome.[3] The most common form of genetically-defined, hereditary CRC, is Lynch syndrome (LS), which accounts for approximately 3.3% of these tumours. Previously referred to as hereditary non polyposis colorectal cancer (HNPCC), Lynch syndrome is inherited as an autosomal dominant disorder.[4, 5] Therefore, if one parent has the disease, there is a 50% chance that each of their children will inherit it. Although characterised by an increased risk of CRC, individuals with Lynch syndrome are also at increased risk of other cancers such as endometrial, ovarian, stomach, small intestine, hepatobiliary tract, urinary tract, brain and skin.[4]

Due to the nature of hereditary syndromes, it is critical to identify affected families for two reasons:

> - Those with a hereditary syndrome and a personal history of CRC have an elevated risk of other non-colorectal cancers as well as a higher risk of metachronous CRC than people without a hereditary syndrome; and
> - Relatives without a personal history of CRC or other cancers have an elevated risk of CRC and other cancers starting at relatively young ages.
>
> *—Page 783 of Ladabaum et al. 2015*[3]

It should be noted that, although Lynch syndrome is characterised by a particular collection of cancers due to a mutation in one of a number of mismatch repair genes, for simplicity, in this report, 'individuals with Lynch syndrome' should be interpreted as "individuals born with Lynch syndrome-causing mutations".

1.1 Condition and aetiology

1.1.1 Aetiology, pathology and prognosis

Lynch syndrome is caused by constitutional pathogenic mutations in DNA mismatch repair genes (MMR). Mismatch repair proteins are involved in recognising and repairing errors during DNA replication.

Although a person with Lynch syndrome still has a functioning MMR system (since they inherit a normal "wild-type" allele from one parent in addition to the mutant allele from the parent with Lynch syndrome) there is a high risk that MMR function will be lost due to somatic mutation of the normal copy of the gene. This loss of MMR function during cell division leads to an inability to repair mutations such as DNA base-base mismatches and small insertions and deletions, eventually resulting in tumorigenesis.[4]

In cells that have lost MMR function, mutations occur all over the genome, but especially in repetitive DNA sequences such as microsatellites.[4] Consequently, in tumours which have

lost MMR function, a large number of mutations are present at microsatellite sequences (as compared to surrounding normal tissue) and this is known as microsatellite instability.

The five currently known genes mutated in Lynch syndrome are[4]:

- MutL homolog 1 (*MLH1*)
- MutS homologs 2 and 6 (*MSH2*, *MSH6*)
- Postmeiotic segregation increased 2 (*PMS2*)
- Epithelial cell adhesion molecule (*EPCAM*)

It should be noted that *EPCAM*, which is not an MMR gene, plays an indirect role in Lynch syndrome.[6] Located upstream of *MSH2*, deletions in the *EPCAM* gene have been shown to result in hypermethylation of the *MSH2* promoter region, leading to a loss of MSH2 expression.[6, 7] In some people it "switches off" the *MSH2* gene which causes an increase in colorectal cancer but not the other *MSH2*-associated cancers. In other individuals, the *EPCAM* deletion stops the *MSH2* gene from working, just like a mutation in the *MSH2* gene itself, in which case all the *MSH2* cancer risks are present.[7] Due to the tissue-dependent levels of EpCAM expression, carriers have a high risk of colorectal carcinoma, whereas extra-colonic cancers are rarely found and the risk for endometrial cancer is reduced.[8, 9]

Colorectal cancers associated with Lynch syndrome develop at a young age and are believed to arise from pre-existing discrete proximal colonic adenomas. Although people with Lynch syndrome develop adenomas at the same rate as individuals in the general population, the adenomas are more likely to progress to cancer. Furthermore, carcinogenesis may progress more rapidly in these patients (in two to three years) than in patients with sporadic adenomas (eight to ten years).[10] Recent evidence also shows that, in addition to the adenoma-carcinoma pathway, colorectal cancers in Lynch syndrome can arise directly from abnormal colonic crypts which have lost MMR.[11] This gives rise to cancers which do not go through an adenomatous polyp phase and therefore has implications for detection and surveillance.[11] The age of onset of CRC in Lynch syndrome varies by MMR gene, but is typically 42 years, i.e., younger than 55 years for the new bowel scope screening gradually being introduced by the NHS, and much younger than the usual 60 to 74 years.[3, 5]

Synchronous colorectal tumours (primary tumours diagnosed within 6 months of each other) and metachronous colorectal tumours (primary tumours occurring more than 6 months apart) are also more common in people with Lynch syndrome.[10] However, there is evidence to suggest that patients with CRC from Lynch syndrome families survive longer than sporadic CRC patients with same-stage tumours, which may be due to a reduced propensity to metastasise. Explanations for this include that immunological host defence mechanisms may be more active in tumours of the MSI phenotype, and that the relatively high mutational load that occurs in tumours with defective DNA repair systems is detrimental to their survival.[12]

1.1.2 Epidemiology

Lynch syndrome affects both men and women, although certain cancers will be specific to each sex, e.g., ovarian or prostate.[13] However, the highest cancer risk is colorectal cancer, where individuals with Lynch syndrome have a risk to age 70 of 33–46%, as opposed to 5.5% and 7.3% (for women and men respectively) in the general population.[1, 5]

The average age of colorectal cancer onset is 44 years in members of families that meet the criteria for Lynch syndrome, whereas for the general population it is 60–65 years.[10] Two thirds of the colorectal cancers occur in the proximal colon (proximal to the splenic flexure) and the risk of a second primary CRC in the patient is high (estimated at 16% within 10 years and 60% if the first CRC was before age 25 years).[14, 15]

The risk of cancer in people with Lynch syndrome varies according to the affected MMR gene, with mutations in *MLH1* and *MSH2* conferring the highest cancer risk (*Table 1*). In individuals ascertained through family history clinics, nearly 90% of mutations are located in *MLH1* and *MSH2,* with approximately 10% in *MSH6* and *PMS2*.[16] Previous estimates of the cumulative risk at 70 years for CRC in *MLH1* or *MSH2* mutation carriers range from 22% to 74%.[16] Mutations in *MSH6* and *PMS2* genes have lower penetrance and different patterns of expression: *MSH6* mutation carriers may have a high risk of endometrial cancer, similar to that in *MLH1* and *MSH2* mutation carriers, but have a later age of onset and a lower risk of CRC.[5]

Table 1: Cancer risk for individuals with Lynch syndrome according to gene

Cancer	**General population lifetime risk**		***MLH1* or *MSH2***		***MSH6***		***PMS2***	
	M	**F**	**Risk (%)**	**Mean age of onset**	**Risk (%)**	**Mean age of onset**	**Risk (%)**	**Mean age of onset**
Colon	7.27	5.47	40-80	44-61	10-22	54	15-20	61-66
Endometrium	-	2.44	25-60	48-62	16-26	55	15	49
Stomach	1.51	0.76	1-13	56	≤3	63	*	70-78
Ovary	-	1.95	4-24	42.5	1-11	46	*	42
Hepatobiliary tract	0.5		1.4-4	50-57	NR	NR	*	NR
Urinary tract			1-4	54-60	<1	65	*	NR
Small bowel	0.01		3-6	47-49	NR	54	*	59
Brain/CNS	1.35	1.37	1-3	~50	NR	NR	*	45
Sebaceous neoplasms			NR	NR	NR	NR	NR	NR
Pancreas	1.44	1.38	NR	NR	NR	NR	NR	NR

Key: CNS, central nervous system; F, female; M, male; NR, not reported
Sources: Cancer Research UK,[1] National Comprehensive Cancer Network (NCCN),[17] and Bonis et al. (2007)[18]

It is interesting to note that the pattern of the cancer sites associated with Lynch syndrome has changed over time. In the first family with the syndrome, gastric and endometrial cancer were the most common cancers whereas in the generations of the same family described by Lynch in 1971 colorectal cancer was the most frequent tumour.[19] Also current differences in expression of the Lynch syndrome between families from Western countries compared to families from the Far East reflect the variation in incidence of cancers in the respective populations.[19] This and more recent work confirms the importance of lifestyle and environmental effects in Lynch syndrome and strengthens the opportunity to give directed advice to those known to have Lynch syndrome.[20]

1.1.3 Incidence and/or prevalence

There were 34,024 new cases of colorectal cancer diagnosed in England in 2014, 18,789 men and 15,236 women.[21] Lynch syndrome accounts for approximately 2% to 3% of cases of CRC with the population prevalence is estimated at 1 in 440.[3, 22] However, according to Hampel, individuals with Lynch syndrome are grossly underdiagnosed. They estimate that the population incidence of Lynch syndrome in the U.S. is approximately 1 in 370, which is based on the 2.8% incidence of Lynch syndrome among newly diagnosed colorectal cancer patients and the 5% lifetime risk for colorectal cancer.[23] As the penetrance of a mutation in the mismatch repair genes for colorectal cancer is approximately 50%, the incidence of Lynch syndrome in the general population is about double the incidence among colorectal cancer patients, or $0.028 \times 0.05 \times 2 = 0.0028$, which is 1 in 370 individuals.[23] On this basis, an estimated 175,000 people in the UK have Lynch syndrome and this leads to over 1,100 colorectal cancers per year across the UK.

Although Lynch syndrome has no known racial proclivity, population-specific mutations are well-described, e.g., in Finnish and Swedish populations. Colorectal cancer rates in the Ashkenazi Jewish population are disproportionately high and although neither Lynch syndrome nor classic familial adenomatous polyposis (FAP) are more common in Ashkenazim than in the general population, both have a connection to individuals of Ashkenazi Jewish heritage.[10] A specific founder mutation in the *MSH2* gene is found in 2-3% of all colorectal cancers in Ashkenazi Jews younger than 60 years. This mutation is rarely found in the non-Ashkenazi population. In Ashkenazi individuals in whom colorectal cancer is diagnosed at age 40 years or younger, 7% have been found to carry this mutation. Conversely, the mutation is found in less than 1% of Ashkenazim persons in whom colorectal cancer is diagnosed after age 60 years.[10]

1.1.4 Impact of health problem

In terms of the impact of Lynch syndrome on an individual, they may develop several characteristic clinical and pathological features[19]:

- Associated cancers: cancer of colorectum, stomach, ovary, ureter／renal pelvis, brain, small bowel, hepatobiliary tract, skin (sebaceous adenoma)
- Development of cancer at an early age
- Development of multiple cancers
- Features of colorectal cancer: predilection for proximal colon, improved survival, multiple colorectal cancers, poorly differentiated tumours and Crohn's-like infiltration of lymphocytes
- Features of adenomas: the numbers vary from one to a few, increased proportion of adenomas with a villous growth pattern, high degree of dysplasia, apparent rapid progression from adenoma to carcinoma

Frequent colonoscopy with polypectomy decreases the mortality of CRC in patients with Lynch syndrome.[3, 5] The current screening protocol recommended by the Mallorca Group of InSiGHT is for colonoscopy every 1–2 years starting from age 25 years.[19] Unfortunately, there is currently no proven surveillance regime for women at risk of endometrial or ovarian cancer.[19] However, prophylactic hysterectomy and oophorectomy after childbearing is complete nearly eliminates the risk of endometrial and ovarian cancer in women with Lynch

syndrome, but the potential effectiveness and place of such prophylactic surgery is now open to reconsideration given that the mortality from endometrial and ovarian cancer in Lynch syndrome is not as high as previously thought.[3, 15]

The risk of gastric cancer in the Lynch syndrome in Western countries is low. Screening by endoscopy has been suggested but is unproven, therefore gastric surveillance should only be discussed in those families that have a high incidence of this tumour.[19] Screening of the urothelial tract is also debatable. While urinary tract cancers are most likely with *MSH2* and *MSH6* mutations, patients with *MLH1* and possibly *PMS2* are also at risk, so a pragmatic approach has been to offer yearly urinalysis, urine cytology and renal ultrasound from age 30–35, but only to families in whom these cancers have been recorded. There is now evidence that the presence or absence of urinary tumours in a family does not predict their occurrence in other family members.[19, 24]

1.1.4.1 Genetic testing for relatives

Understandably, there may be considerable anxiety and distress associated with genetic testing for hereditary cancer syndromes. For example, the knowledge of being at increased risk of cancer but not knowing if cancer will actually develop can be particularly concerning. Furthermore, parents of children with Lynch syndrome may express feeling of guilt. Since people may be anxious about many aspects of genetic testing, screening or whether to have risk-reducing surgery, detailed information provided by an experienced clinical geneticist with psychosocial support is essential. Therefore, it is recommended that relatives opting for genetic testing should receive one or more individual pre-test counselling sessions with psychological support throughout the whole testing procedure.[19]

Genetic counselling helps explain what a positive or negative result means and what the implications are for the person and their extended family. It can also help people understand the importance of informing extended family about their risk of having Lynch syndrome and the benefits of being tested (www.macmillan.org, www.lynch-syndrome-uk.org, www.ihavelynchsyndrome.com/). Once people fully understand the implications of being diagnosed with Lynch syndrome for them and their family, the associated anxiety may substantially reduce. Studies evaluating psychological distress of genetic testing for Lynch syndrome showed that immediately after disclosure of the test result, distress significantly increases, but decreases again after 6 months.[19] Long-term studies have demonstrated that post-result increases in distress return to baseline by 1–3 years.[19]

1.1.4.2 Surgical management

An individual with Lynch syndrome who does not undergo a partial or total colectomy after the first mass is diagnosed as malignant has an estimated 30-40% risk of developing a metachronous tumour within 10 years and a 50% risk within 15 years. It is also now known that the risks from age 40 to 70 years of a metachronous colorectal cancer in those who have had surgery for an initial primary CRC are 46% for *MLH1*, 48% for *MSH2*, and 23% for *MSH6* mutation carriers.[15] This compares to the risks in the general population of 3% in 10 years and 5% within 15 years.[10] Furthermore, due to the risk of a synchronous tumour in individuals with Lynch syndrome, the complete colon should be visualised before resection. In view of this substantial risk, it is possible that a subtotal colectomy instead of a segmental resection might be the preferred treatment in patients from Lynch syndrome families with a primary tumour. Vasen et al. (2013) also suggest that a colectomy with ileorectal

anastomosis may be considered if colorectal cancer is detected in a young patient participating in a surveillance program.[19]

Prophylactic surgery may also be an option for certain extra-colonic cancers, for example, hysterectomy and oophorectomy after childbearing is complete nearly eliminates the risk of endometrial and ovarian cancer in women with Lynch syndrome.[25]

1.1.4.3 Chemotherapy

Fluorouracil based regimes represent the current gold standard in adjuvant chemotherapy for bowel cancer. In contrast, in vitro studies indicate that mismatch repair-proficient cells treated with 5-FU grow more slowly than mismatch repair deficient cells, suggesting that a competent mismatch repair system is a critical condition for 5-FU cytotoxicity.[19] Clinical studies on the efficacy of 5-FU in MSI-H colon cancer are, however, contradictory.[19] A recent systematic review and meta-analysis did not find evidence that chemotherapy response was determined by MSI status,[26] but it is understood that individuals with Stage II colorectal cancer may receive MSI testing or MMR IHC to aid clinical decision making. Stage II tumours showing MSI which are MMR-deficient are usually not treated with (5-FU-based) chemotherapy, since it is associated with toxicity in some patients and is believed to be of marginal clinical benefit.

There is also increasing evidence to show that MMR-deficient cancers respond especially well to PD-1 inhibitors.[27]

1.1.4.4 Chemoprevention

There is evidence to suggest that Lynch syndrome may be susceptible to environmental manipulation, as demonstrated by the decrease in the incidence of gastric cancer and perhaps also by the apparent differences in penetrance between men and women.[19] This is supported by investigations into the role of aspirin in bowel cancer prevention. Several large studies have demonstrated that aspirin reduces the risk of bowel cancer in the general population, although the mechanisms are unknown.[4] A recent study showed that a daily dose of aspirin reduced the incidence of CRC in carriers of Lynch syndrome after 56 months' follow-up.[28] Furthermore, the Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2) trial of aspirin prophylaxis in Lynch syndrome has demonstrated that aspirin treatment for up to 3 years reduces, a decade later, the overall incidence of Lynch syndrome-associated cancers, including CRC, by 63%.[4, 28]

Developments of vaccines directed at tumours with MSI are also showing promise that this may be an especially good way of preventing otherwise un-addressable Lynch syndrome-associated cancers with a poor prognosis, and potentially any cancer in the general population with MSI.[15]

1.1.5 Measurement of disease

In current practice, testing for Lynch syndrome in people with colorectal cancer is targeted using the Amsterdam criteria and Revised Bethesda Guidelines (*Table 2*). The Amsterdam criteria were originally developed to identify Lynch syndrome for research studies and the Bethesda guidelines were developed to identify patients with colorectal cancer for evaluation for MMR deficiency through tumour testing. Both guidelines use criteria mainly based on family cancer history and age at onset.

Table 2: Criteria used to assist diagnosis of Lynch syndrome

Amsterdam II criteria	Revised Bethesda guidelines
All criteria must be met	*Only 1 criterion must be met*
At least three separate relatives with CRC or a LS-associated cancer	CRC diagnosed in a patient aged < 50 years
One relative must be a FDR of the other two	Presence of synchronous, metachronous colorectal or other LS-related tumours, regardless of age
At least two successive generations affected	CRC with MSI-H phenotype diagnosed in a patient aged < 60 years
At least one tumour should be diagnosed before the age of 50 years	Patient with CRC and a FDR with a LS-related tumour, with one of the cancers diagnosed at age < 50 years
FAP excluded in CRC case(s)	Patient with CRC with two or more FDRs or SDRs with a LS-related tumour, regardless of age
Tumours pathologically verified	

Key: CRC, colorectal cancer; FAP, familial adenomatous polyposis; FDR, first-degree relative; LS, Lynch syndrome; MSI-H, microsatellite instability – high; SDR, second-degree relative
Source: Snowsill et al. 2014[4]

These methods are unlikely to be sensitive enough to detect all patients with Lynch syndrome because family history is not always reliable or available, and some people with Lynch syndrome may not meet all the criteria. Furthermore, there is currently no NICE guidance on the population to be tested or the testing strategy for Lynch syndrome and as a result there is considerable variation in clinical practice.

1.2 Description of technologies under assessment

1.2.1 Summary of technology

Overall, the majority of colorectal tumours from individuals with Lynch syndrome genes have two distinguishing characteristics and therefore the diagnostic technologies focus on these aspects:

- Microsatellite instability – PCR-based MSI testing as carried out by UKAS-accredited regional genetics laboratories using validated in-house tests (including the Promega MSI Analysis System, which is licensed for research use only).
- Loss of expression or reduced levels of the mismatch repair proteins in the tumour as compared to normal tissue – Immunohistochemical testing for MMR proficiency using antibodies for MMR proteins.

1.2.1.1 Tumour-based tests

1.2.1.1.1 Microsatellite instability (MSI) testing

As previously mentioned, microsatellite instability refers to the variety of patterns of microsatellite repeats observed when DNA is amplified from an MMR-deficient tumour as compared with DNA amplified from surrounding normal colonic tissue.[4] Repetitive mono- or dinucleotide DNA sequences (microsatellites) are particularly vulnerable to defective MMR.[18]

Microsatellite instability testing involves polymerase chain reaction (PCR) amplification of standardised DNA markers from a tumour tissue sample and a matched (i.e., from the same patient) healthy tissue sample. The tissue samples must be microdissected, where a microscope is used to aid dissection, before DNA is extracted, amplified and observed on a DNA fragment length analyser. MSI is indicated where DNA extracted from tumour tissue displays additional peaks on the analyser output (i.e., microsatellite markers of a different size), in comparison with normal tissue DNA. Tumour samples with microsatellite marker sizes identical to those seen in non-tumour tissue DNA are considered MSS (microsatellite stable).

Laboratories may use a panel of 10 or more markers and, more recently, a commercially available kit based on five mononucleotide markers has become popular as mononucleotide microsatellites may be the most sensitive markers for use in detecting MSI.[29] *Table 3* lists examples of some panels used.

Table 3: Panel of markers for MSI testing

Panel	Mononucleotide	Dinucleotide	Other	Notes
Bethesda/NCI[30]	BAT-25, BAT-26	D2S123, D5S346, D17S250		If only dinucleotide repeats are mutated, a test secondary panel of microsatellite markers with mononucleotide markers to exclude MSI-Low
10-marker panel[31]	BAT-25, BAT-26, BAT-40, MYCL, ACTC, BAT-34C4	D5S346, D17S250, D18S55, D10S197		
NCI suggested markers for secondary panel[30]	BAT-40, MYCL			
Promega MSI Analysis System v1.2[32]	BAT-25, BAT-26, MONO-27, NR-21, NR-24		Penta C, Penta D	

Key: NCI, the National Cancer Institute

Instability in 30% or more of the markers is considered MSI-H, less than 30% MSI-L and no shifts or additional peaks MSS. However, if instability is observed at any mononucleotide markers, MSI may be diagnosed. For this reason, MSI testing is moving to a smaller panel of mononucleotide markers, making the process more efficient and cheaper. MSI-H is associated with Lynch syndrome, but is also present in around 10–15% of sporadic cancers.[19]

There is ongoing debate as to whether MSI-L, which appears to be more common for *MSH6* mutations, should be considered as an indication of microsatellite instability. As a result, some studies do not report MSI-L separately and include it with either MSS or MSI-H. This obviously provides a challenge when comparing studies. There is also some heterogeneity in the composition of microsatellite markers in MSI panels (both in the nature and number of markers), which may lead to differences in test performance and/or threshold effects.

In the UK, PCR based microsatellite instability testing is carried out by UKAS (United Kingdom Accreditation Service) accredited regional genetics laboratories using in-house tests which are internally validated within the laboratories (including the Promega MSI Analysis System, which is licensed for research use only).

Personal communication from IMF indicates that MSI test failures occur in a small proportion of tests (around 5%), largely due to technical challenges surrounding the collection of sufficient DNA out of poorly-fixed tumour tissue.

1.2.1.1.2 MMR Immunohistochemistry

MMR immunohistochemistry (IHC) tests for the presence or absence of MMR proteins in colorectal tumour cells. Antibodies for the MMR proteins are used to stain both tumour cells and non-tumour cells (as internal controls). If nuclear staining is present for all MMR proteins, this suggests the MMR system is intact. In contrast to MSI, a number of patterns of abnormal MMR expression are seen in Lynch/HNPCC tumours (*Table 4*).

Table 4: Underlying causes of microsatellite instability in colorectal and endometrial cancers in genetics clinic patients, by associated pattern of MMR IHC abnormality

MMR mutation	Loss of (or otherwise abnormal) expression by IHC				Overall
	MLH1 (alone, or in combination with PMS2)	MSH2 (alone, or in combination with MSH6)	MSH6 (alone)	PMS2 (alone)	
Lynch syndrome					
Constitutional *MLH1* mutation	11.8%			2.0%	**14%**
Constitutional *MLH1* methylation[a]	0.4%				**0.4%**
Constitutional *MSH2* mutation		14.2%	0.4%		**15%**
Constitutional *EPCAM* mutation		2.0%			**2.0%**
Constitutional *MSH6* mutation		0.8%	10.2%		**11%**
Constitutional *PMS2* mutation				5.9%	**5.9%**
Not Lynch syndrome					
Acquired *MLH1* methylation	24.0%				**24%**
Acquired *MLH1* mutation	6.7%				**6.7%**
Acquired *MSH2* mutation		2.4%			**2.4%**
Unexplained	10.2%	5.9%	1.6%	1.6%	**19%**
Total	53%	25%	12%	9%	**100%**

Note: [a] Constitutional *MLH1* epimutations are not universally recognised as being Lynch syndrome-causing mutations, since these are not always inherited[33]
Source: Frayling and Arends (2015)[34]

Table 4 highlights that for some individuals with abnormal MMR IHC in the tumour, no germline mutation can be found. Somatic mutations in MMR genes can occur (with attendant loss of MMR protein expression) as secondary events in other CRC predisposition syndromes which can mimic Lynch syndrome, such as inherited mutations in *MUTYH* or *POLD1*.[35, 36]

It is also important to realise that a small proportion of possibly Lynch-related tumours do not exhibit any abnormality on analysis by IHC, even though they have lost MMR function as demonstrated by microsatellite instability (MSI), and this may be due to mutations that allow expression as a stable protein with nuclear localisation and an intact epitope but which is functionally inactive.[37]

IHC panels may use two MMR antibodies (either MLH1 and MSH2, or MSH6 and PMS2) or four antibodies (MLH1, MSH2, MSH6 and PMS2). However, a panel with only MLH1 and MSH2 antibodies is unlikely to detect MMR deficiency in MSH6 and PMS2, will be expected to have lower sensitivity than a four body panel, and will not be able to fully diagnose the pattern of abnormal expression seen in all tumours.[34] Furthermore, the heterodimeric association of proteins such that loss of MLH1 expression is almost always accompanied by loss of PMS2 expression, and MSH2 with loss of MSH6 acts as a very useful confirmatory finding, especially in colorectal cancers which are prone to suboptimal fixation.[34, 37] A four-antibody panel is recommended in the UK.[38]

Test failure is a recognised issue with IHC, and is usually due to incomplete tissue fixation (a common problem with colorectal cancers). Palomaki et al. (2009) counted six test failures across a number of studies (total 136 patients), corresponding to a failure rate of 4.4%.[39]

1.2.1.1.3 *BRAF* V600E mutation testing and *MLH1* methylation testing

Around 10 to 15% of sporadic CRC show MSI-H and in the vast majority of these, this will be due to acquired promoter methylation of the *MLH1* gene leading to loss of MLH1 protein expression. However, a small proportion of sporadic MSI-H CRCs will occur due to loss of *MSH2*, *MSH6* or *PMS2* and germline *MLH1* hypermethylation will be observed in a very small number of some colorectal cancers due to Lynch syndrome.[37, 40] Approximately half of the sporadic CRCs with MSI-H will also have a *BRAF* V600E mutation – this is a specific mutation in the *BRAF* gene which almost never occurs in tumours arising in Lynch syndrome.

Therefore, testing for *MLH1* promoter methylation and *BRAF* V600E mutation represent ways of distinguishing sporadic CRC from Lynch syndrome in a proportion of MLH1-negative tumours.[37]

1.2.2 Identification of important sub-groups

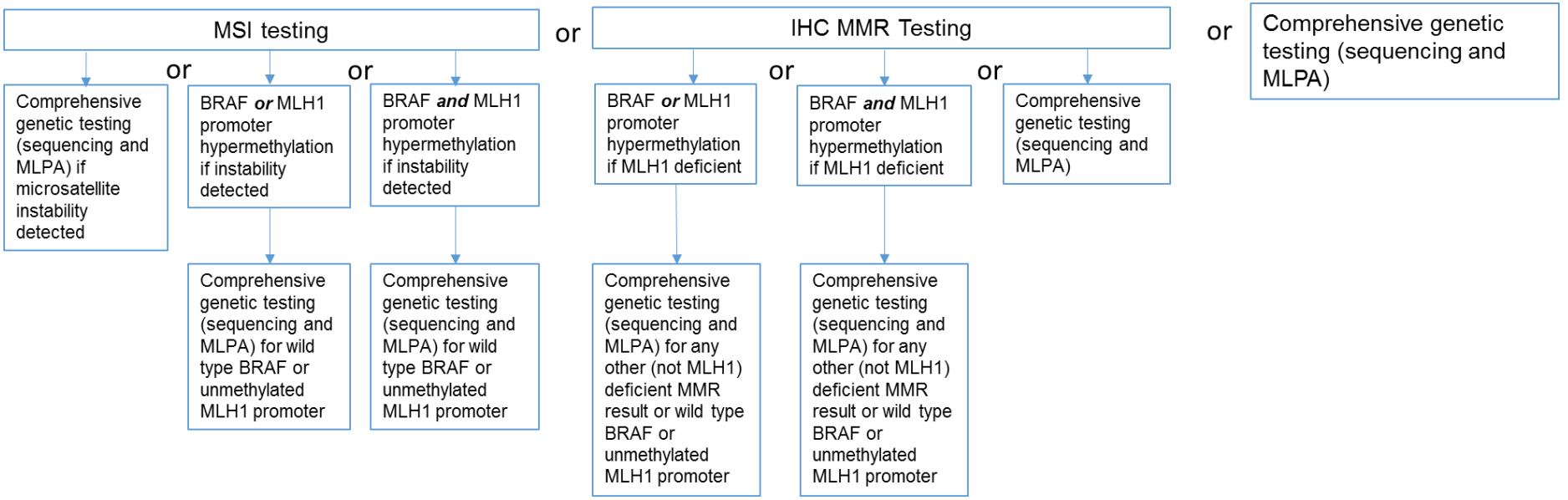
First degree relatives of a Lynch syndrome mutation carrier have a 50% risk of inheriting the mutation. Therefore where the familial mutation has been identified, cascade testing should be offered to the first and second and, when possible, third-degree biological relatives. Recent studies showed that the uptake of genetic testing in families with Lynch syndrome varied from 43% in the US, 57% in the Netherlands to 75% in Finland.[19] Suggested reasons for this variation include differences in the study setting or fundamental differences between the health care and social security systems.

Since colorectal cancer in an individual with Lynch syndrome is likely to be diagnosed at a younger age, the prevalence of Lynch syndrome in people with colorectal cancer will vary across age groups. For example, the prevalence of Lynch syndrome falls from 8.4% at 50 years, to 5.7% at an 60 years and 3.8% at 70 years.[4] This is because the incidence of CRC in the general population rises more rapidly than the incidence of CRC in people with Lynch syndrome. The total annual incidence of cases of CRC in England increases from 2,107 at 50 years, to 5,880 at 60 years and 13,823 at 70 years.[21]

1.2.3 Current usage in the NHS

Microsatellite instability (MSI) testing for the purpose of identifying Lynch syndrome is currently only done in people considered to be at high risk of having Lynch syndrome. That is, people with a family history of cancer and/or who are younger than 50 years old at the onset of cancer. For cancer where there is no suspicion of Lynch syndrome, MSI or IHC testing may yet be conducted to inform prognosis or to guide therapy. Expanding diagnostic testing to all people with colorectal cancer population and identification of families who could benefit from cascade genetic testing may lead to increased surveillance and consequently improved patient outcomes through earlier diagnosis and treatment. Currently, testing for Lynch syndrome may occur via a number of different strategies (*Figure 1*).

Figure 1: Diagnostic test strategies for Lynch syndrome



1.2.4 Anticipated costs associated with the intervention

MSI and IHC are both tumour based tests, the costs of which need to include the cost of preparing the sample, analysing the sample and reporting the test results; as well as costs of administration, transportation, additional wear and tear on machinery, training time and repeat tests. As a Lynch syndrome diagnosis cannot be confirmed with just MSI or IHC, the cost of downstream testing also impacts the overall cost of the interventions, as well as the number of downstream tests that will be run as a result MSI or IHC testing indicative of Lynch syndrome.

In Snowsill et al. (2014), listed costs for constitutional DNA tests were assumed to be all-inclusive: all laboratory, processing and transportation costs were assumed to be accounted though core funding.[4] It is not clear if this will continue with an increase in number of tests requested. There is also the additional factor that gene sequencing costs in particular have reduced in the last two years in UK regional genetics laboratories with the increasing introduction of Next Generation Sequencing (NGS).

Costs of the diagnostic tests are detailed further in *Diagnostic tests* (*page 204*). Unit costs for MSI and IHC are estimated to be £178 and £210, respectively. This is equivalent to ~£7,000,000 (without additional tests) for a cohort of ~34,000 individuals with newly diagnosed colorectal cancer,[21] increased from an estimated ~£2,000,000 for the same cohort under a no reflex diagnostic testing strategy (assuming that some people with CRC will receive MSI and IHC for therapeutic/prognostic purposes). However, this total cost is unlikely to remain constant, as the number of individuals with newly diagnosed CRC, and number of families with Lynch syndrome identified (for whom MSI and IHC diagnostic testing will be unnecessary) are likely to differ in the future.

1.3 Comparators

1.3.1 Constitutional DNA tests

The gold standard for diagnosis of Lynch syndrome is comprehensive screening for constitutional mutations in the MMR genes and *EPCAM*. This screening is conducted using a DNA sequencing method to detect point mutations, small insertions and deletions and MLPA to detect large structural DNA abnormalities, such as genomic deletions, duplications and rearrangements.[4] Sequencing is usually performed on lymphocytic DNA from a blood sample. The various forms of mutations are described below:

- Missense - A change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene
- Nonsense - A change in one DNA base pair that results in a premature signal to stop building a protein. This type of mutation results in a shortened protein that may function improperly or not at all
- Insertion - Changes the number of DNA bases in a gene by adding a piece of DNA. As a result, the protein made by the gene may not function properly
- Deletion - Changes the number of DNA bases by removing a piece of DNA. Small deletions may remove one or a few base pairs within a gene, while larger deletions can remove an entire gene or several neighbouring genes. The deleted DNA may alter the function of the resulting protein(s)

- Duplication - Consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein
- Frameshift mutation - Occurs when the addition or loss of DNA bases changes a gene's reading frame. A reading frame consists of groups of three bases that each code for one amino acid. A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions and duplications can all be frameshift mutations
- Splice site - Causes abnormal mRNA processing, generally leading to in-frame deletions of whole exons or out-of-frame mRNA mutations leading to nonsense-mediated decay of mRNA. Mutations may be located deep in intronic sequences
- Promoter – Mutations [occurring] in the controlling region of a gene [the promoter] leading to its non-expression. Epigenetic mutations [in the promoter], i.e. abnormal methylation of CpG sites[,] may give rise to the same effect

—Adapted from Genetics Home Reference[41]

Other techniques, as listed in *Table 5*, may be found in older studies.

Although comprehensive screening for constitutional mutations should accurately detect the majority of known Lynch syndrome-causing mutations, there are some occasions where a novel mutation may be identified which is of uncertain significance. Since this variant or mutation cannot be demonstrated to be pathological or non-pathological, it is not possible to make a diagnosis or recommendations for management, such as colorectal surveillance. However, a major advance has been the establishment by the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) of an internationally-recognised reference database together with a multi-disciplinary team of experts to maximise the number of mutations interpreted as of either clinical consequence or which are innocuous, thus minimising the number in the 'uncertain' bracket. Before this work, 58% of the 12,006 mutations listed were unclassified variants (UV), a proportion which has been reduced to 32%, i.e., those mutations which now fall into the category of 'variants of uncertain significance' (VUS), otherwise known as Class 3.[42] Hence, this now enables a Class 3 the variant of uncertain significance (VUS) to be pursued by testing for the variant in other family members with Lynch syndrome-related cancers or by testing stored tumour tissue for MMR deficiency.

Table 5: Genetic testing in Lynch syndrome Test Description Comments High-output screening techniques

Test	Description	Comments
High-output screening techniques	Single-strand conformational polymorphism (SSCP) Conformation-sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-performance liquid chromatography (DHPLC)	These methods use the change in chemical properties of altered DNA to differentiate from normal DNA (now considered obsolescent/obsolete in the UK)
DNA sequencing	Can be used following high-output screening technique or as primary approach when directed by IHC patterns	The main method used in the UK for detecting most MMR gene mutations. However, it does not reliably detect deletions or rearrangements.
Methods to detect large structural DNA abnormalities	Multiplex ligation-dependent probe amplification (MLPA)	MLPA is the preferred technique in the UK. Large structural DNA abnormalities are an important cause of LS (5–25% of cases, depending on the gene) but are not generally detected by high-output screening techniques or DNA sequencing. MLPA, which involves measurement of the relative copy number of DNA sequences, has evolved to become a standard approach for analysing MMR genes for deletions4
Conversion analysis	Only a single allele is analysed at a time. This can increase the yield of genetic testing but is technically complicated, expensive and not widely available	

Source: Snowsill et al. (2014)[4]

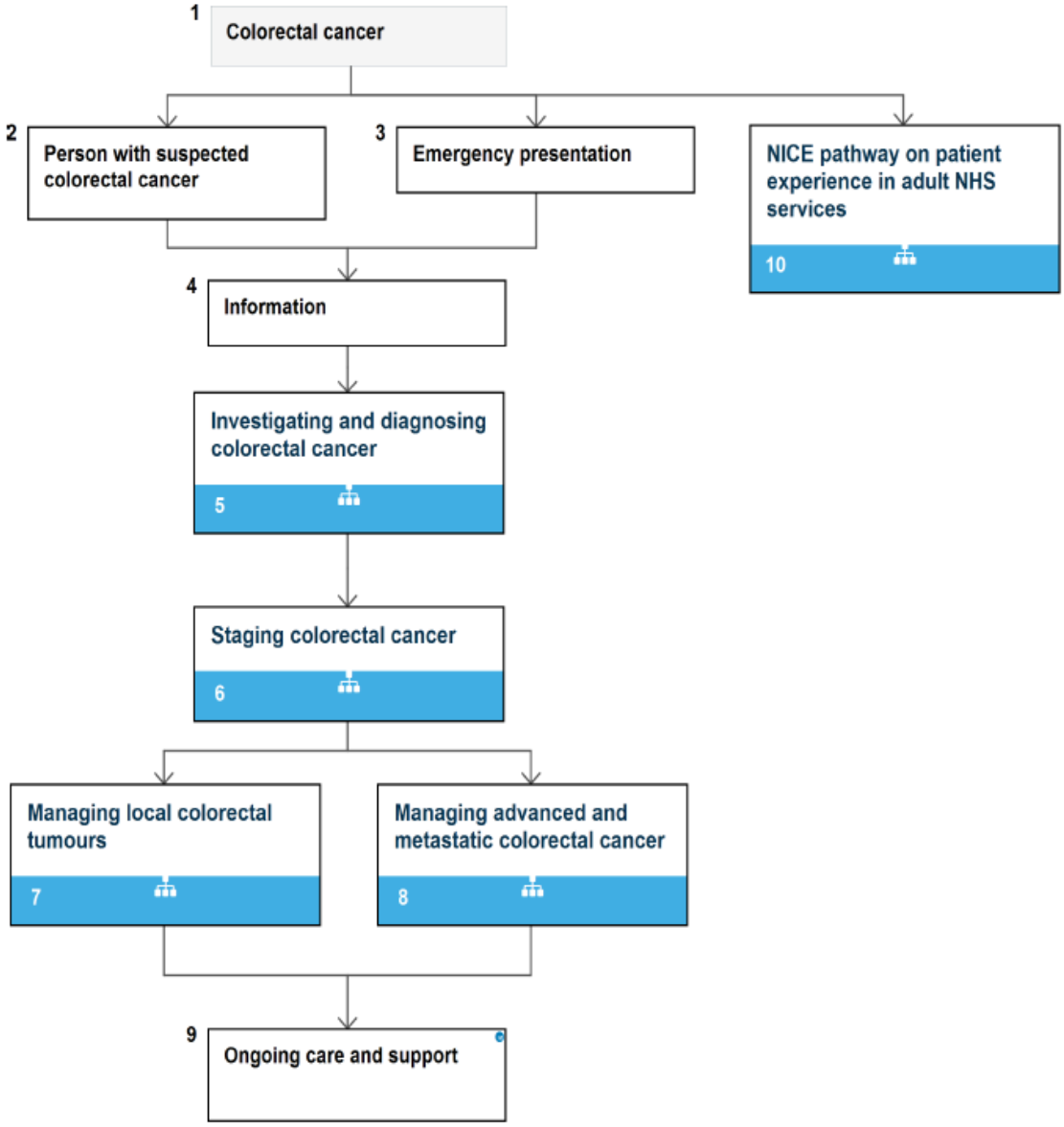
1.4 Care pathways

There is currently no NICE guidance on the diagnosis and management of Lynch syndrome, however the diagnosis and management of Lynch syndrome is described in several national and international guidelines:

- British Society of Gastroenterology: Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (2010)[43]
- European Guidelines: Revised guidelines for the clinical management of Lynch syndrome (HNPCC) (2013)[19]
- Bethesda Guidelines: Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch syndrome) and Microsatellite Instability (2004)[30]
- Amsterdam II criteria: New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative Group on HNPCC (1999)[44]

In the NHS, colorectal cancer in Lynch syndrome patients is generally treated as per NICE Clinical Guideline 131: Colorectal cancer: The diagnosis and management of colorectal cancer (November 2011) (*Figure 2*).[45]

Figure 2: Overview of pathway for colorectal cancer



Source: NICE clinical pathway for colorectal cancer[45]

The European Society for Medical Oncology guidelines, 'Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up' are also used by clinicians in the NHS to guide treatment decisions. The guidelines state that "MSI/MMR may be useful to identify a small (10%–15%) subset (those with microsatellite instability) of stage II colorectal cancer patients who are at a very low risk of recurrence and in whom the benefits of chemotherapy are very unlikely".[46]

The Royal College of Pathologists (RCPath) minimum dataset (July 2014) for colorectal cancer mandates the use of immunohistochemistry or other testing for molecular features of

Lynch syndrome in colorectal cancer or Lynch associated cancer patients under the age of 50, at the time of diagnosis.[47] However, the results of a 2015 Bowel Cancer UK survey on reflex testing for Lynch syndrome in people diagnosed with bowel cancer under the age of 50 highlighted that there is variability in who receives testing among the Trusts and Health Boards.[48] Ideally, MSI and IHC will usually be conducted on tumour tissue obtained during surgical treatment or via biopsy. A histopathologist selects tissue for testing and performs microdissection for MSI or sectioning and staining for IHC. Microdissected samples for MSI testing are processed by a laboratory genetics centre where PCR-based MSI testing is performed and reported to the histopathologist, who in turn informs the cancer team (usually a consultant colorectal surgeon) along with recommendations for further testing.

If the results of MSI and/or IHC testing are suggestive of Lynch syndrome there may be further tumour tissue based tests ordered (e.g., IHC, *BRAF* V600E mutation testing, *MLH1* methylation testing) or the patient may be referred directly to clinical genetics. At this point, clinical genetics will discuss the findings with the patient, describe Lynch syndrome and take a detailed family history (pre-test genetic counselling). If the genetics team and the patient agree that constitutional MMR mutation testing is appropriate then a blood sample will be sent to laboratory genetics for testing.

If a pathogenic constitutional MMR mutation is not found, or a VUS is found, or the mutation identified is inconsistent with existing findings, the genetics team will provide appropriate counselling and further testing and propose an appropriate management strategy for the patient.

1.5 Outcomes

The accuracy of MSI and IHC testing of tumour tissue for Lynch syndrome has been evaluated against the reference (gold) standard of constitutional genetic testing. Clinically important outcomes relevant to test accuracy include:

- Sensitivity: the probability of detecting Lynch syndrome in someone with Lynch syndrome

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} = \frac{TP}{TP + FN}$$

- Specificity: the probability of not detecting Lynch syndrome in someone without Lynch syndrome

$$\text{Specificity} = \frac{\text{True negative}}{\text{False positive} + \text{True negative}} = \frac{TN}{FP + TN}$$

- Likelihood Ratio (LR) is the likelihood that a given test result would be expected in a patient with the target disorder compared to the likelihood that that same result would be expected in a patient without the target disorder.
    - Likelihood ratio for positive test result (LR+). This is how much more often a positive test occurs in people with compared to those without the disease.

$$LR+ = \frac{\Pr(T+|D+)}{\Pr(T+|D-)} = \frac{\text{Sensitivity}}{1 - \text{Specificity}}$$

- Likelihood ratio for negative test result (LR−). This is how much less likely a negative test result is in people with the disease compared to those without the disease.

$$LR-= \frac{\Pr(T- \mid D+)}{\Pr(T- \mid D-)} = \frac{1 - \text{Sensitivity}}{\text{Specificity}}$$

- Positive predictive value (PPV). This is the probability of someone with a positive result actually having Lynch syndrome.

$$PPV = \frac{TP}{TP + FP}$$

- Negative predictive value (NPV). This is the probability of someone with a negative test result actually not having Lynch syndrome.

$$NPV = \frac{TN}{TN + FN}$$

- Diagnostic yield (also known as test positivity rate or apparent prevalence). This is the number of positive test results divided by the number of samples.
- Test failure (non-informative test result) rate.

Outcomes relevant to cost effectiveness are:

- Number of individuals with Lynch syndrome receiving Lynch syndrome surveillance.
- Number of individuals with Lynch syndrome not receiving Lynch syndrome surveillance.
- Number of individuals without Lynch syndrome receiving Lynch syndrome surveillance.
- Number of individuals without Lynch syndrome who do not receive Lynch syndrome surveillance.
- Sensitivity and specificity of each diagnostic strategy (as opposed to individual tests).
- Costs of each strategy (discounted and undiscounted). This includes disaggregated costs of diagnosis and outcomes.
- QALYs (discounted and undiscounted).
- Overall survival (and whether censored upon reaching age 100).
- Colorectal cancer-, endometrial cancer- and overall cancer-free survival (and whether censored due to death or reaching age 100).
- Event-free survival (and whether censored upon reaching age 100).
- Number of incident colorectal cancers.
- Number of incident endometrial cancers.
- Number of colonoscopies performed.

2 Assessment of test accuracy

2.1 Methods for reviewing effectiveness

The diagnostic accuracy of molecular MSI testing and MMR immunohistochemistry (each with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing) was assessed by a systematic review of the research evidence. The review was undertaken following the principles published by the University of York Centre for Reviews and Dissemination (CRD).[49]

2.1.1 Identification of studies

The following bibliographic databases were searched to identify studies: MEDLINE, MEDLINE In-Process & Other Non-Indexed Citations, Embase and the Health Management Information Consortium (all via Ovid); Web of Science (including conference proceedings, via Thomson Reuters); the Cochrane Database of Systematic Reviews, CENTRAL and HTA (all via the Cochrane library). The search strategies were developed by an information specialist (SB), and comprised of population terms for Lynch syndrome or hereditary non-polyposis colorectal cancer and intervention terms for MSI or IHC. Methodological filters for test accuracy studies were not used to limit the study designs retrieved as these have been shown to reduce sensitivity.[50] Search results were limited by date from 2006 to current (searches were run in February 2016) and to English language studies. The full search strategies for each database are reproduced in *Appendix 1*.

The search results were exported to Endnote X7 (Thomson Reuters, NY, USA) and de-duplicated using automatic and manual checking.

In order to identify relevant studies published before 2006, Bonis et al. (2007) was screened.[18] In addition, Palomaki et al. (2009), Snowsill et al. (2014) and Vasen et al. (2013), as well as any other systematic reviews identified by the bibliographic database searches, were used to source relevant studies published before 2006 and additional studies published after 2006.[4, 19, 39] For the purpose of this review, a systematic review was defined as one that has: a focused research question; explicit search criteria that are available to view; explicit inclusion/exclusion criteria; a critical appraisal of included studies, including consideration of internal and external validity of the research; and a synthesis of the included evidence (narrative or quantitative).

Items included after full-text screening were forward citation chased using Scopus (Elsevier). The reference lists of included studies were also screened for any other relevant studies.

Relevant studies were then identified in two stages. First, titles and abstracts returned by the search strategy were examined independently by two researchers (HC and TJH) and screened for possible inclusion, using pre-specified inclusion and exclusion criteria (see *Section 2.1.2*, *page 68*). Disagreements were resolved by discussion. Full texts of studies included at the title and abstract screening stage were obtained, as were full texts of studies identified from systematic reviews, and from forward and backward citation chasing. Two researchers (HC and TJH) independently examined full texts for inclusion or exclusion. Disagreements were again resolved by discussion.

2.1.2 Inclusion and exclusion criteria

2.1.2.1 Population

Studies of individuals with colorectal cancer were included (CRC). This included fresh samples taken from people who were newly diagnosed with CRC or samples which had been retained in storage.

The unit of assessment was individual patients. If results were presented according to individual cancers (e.g., when patients have multiple primary colorectal malignancies) then, where possible, the earliest colorectal cancer tested with an index test was used as the unit of assessment.

Studies in which clinical or family history criteria were used to select colorectal cancer patients were eligible for inclusion under certain circumstances (see *Section 2.1.2.5*, *page 69*).

2.1.2.2 Index tests

The index tests to be considered were:

- Molecular MSI testing, with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing;
- MMR immunohistochemistry, with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing.

Studies in which *BRAF* V600E and/or *MLH1* methylation tests were only performed on certain patients according to their MSI or IHC test results were eligible for inclusion.

Studies were eligible for inclusion if one or more index test was assessed versus a reference standard.

2.1.2.3 Reference standard

The reference standard was constitutional MMR mutation testing (for abnormalities which provide a genetic diagnosis of Lynch syndrome) which, as a minimum, included DNA sequencing and MLPA (or another appropriate technique for detecting large genomic abnormalities). Other appropriate techniques were Southern blot analysis, gene-targeted array-based comparative genomic hybridization (aGCH), and next generation sequencing (NGS). However, NGS alone (i.e., without MLPA) was accepted as an includable technique for detecting large genomic abnormalities only if the study described or cited peer-reviewed methodology for identifying structural variants in Lynch syndrome based on output data. If no such methodology was described it was assumed NGS would not detect structural variants and would not be an includable technique. Studies were eligible for inclusion if MLPA, southern blot, aGCH or NGS (as described above) was only conducted when sequencing found no clearly pathogenic mutations. Studies in which IHC results directed the MMR genes to be tested were eligible for inclusion (e.g., if *MLH1* was not tested when only MSH2 and MSH6 proteins were absent on IHC).

Unless the aim of a study was to investigate the test accuracy of an index test in individuals with mutations in a particular MMR gene, studies must have tested *MLH1*, *MSH2* and *MSH6* as a minimum (unless IHC results directed otherwise).

2.1.2.4 Outcomes

The outcomes assessed for index tests were:

- Sensitivity;
- Specificity;
- Likelihood ratio for positive test result (LR+);
- Likelihood ratio for negative test result (LR−);
- Positive predictive value (PPV);
- Negative predictive value (NPV);
- Accuracy or concordance with reference standard: the proportion of test results correctly identified by the test, i.e., the rate of agreement with the reference standard

$$\text{Accuracy} = \frac{\text{True positive} + \text{True negative}}{\text{Total number of subjects}} = \frac{TP + TN}{TP + FP + FN + TN}$$

- Diagnostic yield (also known as test positivity rate or apparent prevalence);
- Test failure (non-informative test result) rate.

2.1.2.5 Study design

Single-gate diagnostic studies with random or consecutively recruited participants were considered the optimal design for evaluating test accuracy of MSI and IHC and were, therefore, eligible for inclusion. Two-gate diagnostic studies were also included.

Studies were included if all participants received the index test(s) and the reference standard. Studies which recruited a representative sample of all colorectal cancer patients, but did not apply the reference standard to all patients, were included if the reference standard was applied to all patients testing positive for one or more index test *and* to a representative (e.g., random) sample of patients testing negative for all index tests.

Studies which limited recruitment to high-risk populations (except by applying an age limit to an otherwise population-based sample) were only included to estimate sensitivity, and only if the index test(s) and reference standard had been applied to all participants.

2.1.3 Data abstraction strategy

Data were extracted by one reviewer (TJH) using a standardised data extraction form (*Appendix 2*) and checked by a second reviewer (HC). Disagreements were resolved by discussion, with involvement of a third reviewer if necessary. Data were then transferred to standardised tables.

2.1.4 Critical appraisal strategy

The methodological quality of the studies was assessed according to criteria specified by Phase 3 of the QUADAS-2 tool (*Appendix 3*).[51] This was done alongside, and in the same form as the data extraction (*Appendix 2*).

Assessments were conducted by one reviewer (TJH) and judgements were checked by a second (HC). Any disagreement was resolved by discussion, with involvement of a third reviewer as necessary.

2.1.5 Methods of data synthesis

The extracted data from each study were analysed in STATA 13: for studies based on high risk samples, the sensitivity of the index test(s), with 95% CIs, was calculated. For studies that were not based on high risk samples (including studies where the population was age-limited), sensitivity, specificity, LR+, LR−, PPV and NPV, with 95% CIs, were calculated, where data permitted. Of these latter studies, those that provided estimates of both sensitivity and specificity had their point estimates plotted in ROC space.

The data extracted from each study, the results obtained from analysing individual studies and the quality assessment for each study are presented in structured tables and in a narrative synthesis. Any possible effects of study quality on the data are discussed.

Data from individual studies were not pooled in meta-analysis; once data from individual studies were sorted into categories (e.g., high-risk population or age limited population; MSI-L defined as positive or defined as negative) there were insufficient methodologically homogenous data sets to enable meaningful data pooling.

2.2 Results

2.2.1 Quantity and quality of research available

The electronic searches retrieved a total of 3,920 unique titles and abstracts. A total of 3844 articles were excluded, based on screening titles and abstracts. The remaining 77 articles were requested as full texts for more in-depth screening.

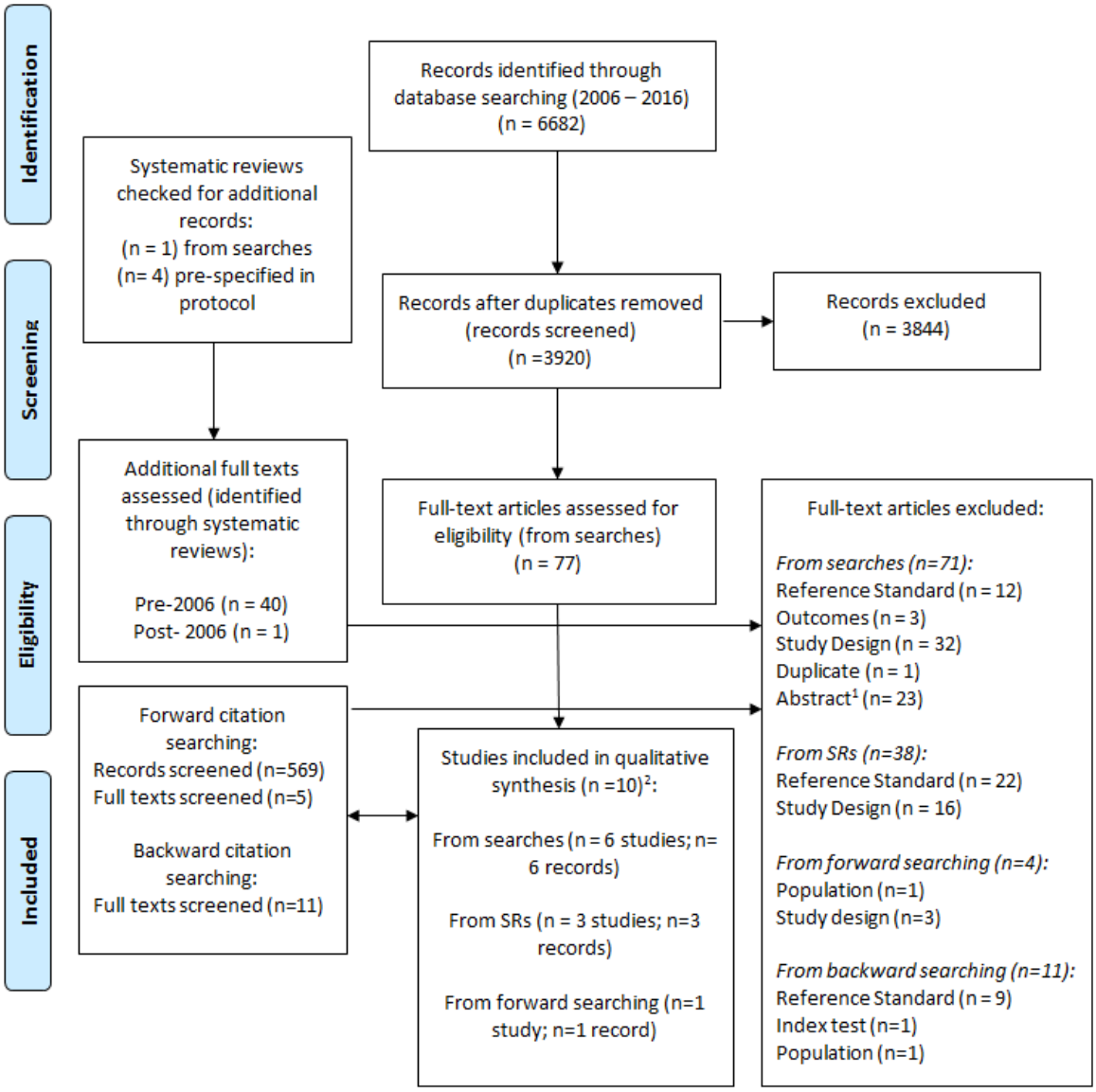
After screening systematic reviews, including those that were pre-specified (Bonis, 2007; Palomaki, 2009; Snowsill, 2014; Vasen, 2013), a further 41 articles were retrieved as full texts for in depth screening.[4, 18, 19, 39]

Of the 118 articles retrieved as full texts (identified from electronic searches and systematic reviews), 109 were excluded. The primary reasons for exclusion were: the study design (n=48), reference standard (n=34), or outcomes (n=3) did not match the review inclusion criteria, the article was a duplicate publication (n=1), or the article was an abstract that had both insufficient information to be included in the review and was unconnected to any of the included studies (n=23). The bibliographic details of studies retrieved as full papers and subsequently excluded, along with the reasons for their exclusion are detailed in *Appendix 4*. The remaining nine studies were included.

After backward and forward citation chasing, a further 16 full text papers were obtained, of which one study was included and 15 were excluded because the reference standard (n=9), study design (n=3), population (n=2), or index test (n=1) did not match the review inclusion criteria. The bibliographic details of these excluded studies, along with the reasons for their exclusion are also given in *Appendix 4*.

In total, therefore, 133 full text articles were assessed, of which 10 studies met the review inclusion criteria. The process of study selection is shown in *Figure 3*. It should be noted that one of the included studies had two distinct samples (a population-based sample and a high-risk sample) and, therefore, had two distinct sets of data. These two samples are treated separately in this review, so although there are 10 included studies, there are, in fact, 11 included populations/data sets. The results from all 11 populations are included in the narrative synthesis.

Figure 3: Summary of the selection process



[1] Abstracts were excluded when they were not linked to an included study and did not provide sufficient methodological information to meet the review inclusion criteria or have data extracted

[2] One of these studies included two distinct populations, both of which are included in this review. Although there are 10 included studies, there are, therefore, 11 included datasets

Adapted from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Key: SR, systematic review

2.2.2 Description of included studies

Four of the included studies reported data from a population-based sample. The study by Poynter et al. (2008) reported data from two populations, one of which appeared to be an unselected CRC population, although this is not entirely clear because participant inclusion criteria were not reported.[31] The other three population-based studies (Barnetson, 2006; Limburg, 2011; Southey, 2005) included CRC populations to which an age-limit had been applied.[52-54] All of these, and the study by Poynter et al. (2008) were single-gate studies.[31]

The remaining studies (including the other sample reported in Poynter, 2008) were based on participants with CRC who were also selected for being high-risk for Lynch syndrome. Five of these studies had a single-gate design (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005).[31, 55-58] The remaining two studies were variations on a two-gate study design (Hendriks, 2003, Okkels, 2012); in these two studies participants with positive reference standard results were recruited but no reference standard negatives were recruited, thus resembling half of a two-gate design from which sensitivity estimates could be obtained.[59, 60] These studies do not, therefore, have two-gates and from this point forward, for clarity, will be referred to as reference standard positive studies.

Table 6 provides a summary of all studies included in the test accuracy review. A narrative summary of the included studies and their population characteristics is provided in *Sections 2.2.2.1* to *2.2.2.3*.

2.2.2.1 Single-gate studies recruiting population-based samples

As mentioned above, the population-based sample included in the study by Poynter et al. (2008) appeared to be completely unselected.[31] The other three population-based studies included in this review were based on CRC populations to which an age-limit had been applied (Barnetson, 2006; Limburg, 2011; Southey, 2005).[52-54] For all three of these studies this limit was for age at diagnosis (rather than age at recruitment) and was <55 years for Barnetson et al. (2006), <50 years for Limburg et al. (2011) and <45 years for Southey et al. (2005).[52-54] These four studies varied in size; the studies by Barnetson et al. (2006) and Poynter et al. (2008) were the largest, recruiting 1,259 participants and 1,061 participants respectively.[31, 52] The studies by Limburg et al. (2011) and Southey et al. (2005) recruited similar numbers of participants (n=195 and n=131 respectively).[53, 54]

With regards to the population characteristics of these four studies, all provided details on the participants' gender and for all studies the ratio of males to females was similar (*Table 7*). Although the study by Poynter et al. (2008) included the only unselected CRC population identified by this review, the age of participants was not reported.[31] The mean or median age for the other three studies ranged from 49.0 (±3.9) years for one of the subgroups in the Barnetson et al. (2006) study (*Table 7*) to 37.1 (range 24 to 42) years for those receiving the reference standard in the Southey et al. (2005) study.[52, 54] These low mean and median ages, which were similar across these three studies, are unsurprising given that all three applied an age-limit to participants for inclusion in the study (Barnetson, 2006; Limburg, 2011; Southey, 2005).[52-54] The four studies all reported a low proportion of participants meeting AMS II criteria, ranging from 0.1% in one of the subgroups in Poynter et al. (2008) to 12% in Southey et al. (2005), with two studies also reporting the proportion of participants meeting RBG (*Table 7*).[31, 54] The specific location of the CRC was reported by Barnetson et al. (2006) and Poynter et al. (2008), but not by Limburg et al. (2011) or Southey et al. (2005), and where possible is given in *Table 7*.[31, 52-54] None of the studies provided details on the ethnicity of participants.

Three of the studies recruiting population-based samples (Barnetson, 2006; Poynter, 2008; Southey, 2005)[31, 52, 54] assessed MSI and IHC, whereas Limburg et al. (2011)[53] assessed only IHC (*Table 6*).

Table 6: Summary of studies included in the review of test accuracy

Study	Participants and selection	N	Ref standard	MSI	MSI Panel	IHC	IHC proteins
Single-gate studies recruiting population-based samples							
Barnetson, 2006[52]	Diagnosed <55yrs of age, consecutive recruitment	Recruited: 1259 RS:870 MSI:352 IHC: 312-328	D-HPLC for *MSH2* and *MLH1*. Noted variants were sequenced (as were 5 *MSH2* exons and 3 *MLH1* exons and all 10 *MSH6* exons). *MLH1* and *MSH2* were assessed for deletions by MLPA.	Y	BAT25, BAT26, D2S123, D5S346, D17S250	Y	MLH1, MSH2, MSH6
Limburg, 2011[53]	Diagnosed <50yrs of age, random recruitment	Recruited: 195 RS:189-195 IHC: 155	Direct sequencing following PCR. Potential variants were confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination. *MLH1* and *MSH2* were assessed for deletions by Southern blot and MLPA.	N	N/A	Y	MLH1, MSH2, MSH6
Poynter, 2008[a; 31]	Recruitment through population-based cancer registries (population-based sample), selection process unclear[b]	Recruited: 1061 RS:726 MSI:1061 IHC: 719	For *MSH2* and *MLH1*: a combined approach of D-HPLC/direct sequencing and multiplex ligation dependent probe amplification (MLPA). For *MSH6*: Direct sequencing in cases with absent immunohistochemical staining of MSH6.	Y	BAT25, BAT26, D5S346, D17S250, BAT40, MYCL, ACTC, Dl 8S55, D1OS197, BAT34C4	Y	MLH1, MSH2, MSH6 PMS2
Southey, 2005[54]	Diagnosed <45yrs of age, random recruitment	Recruited: 131 RS:59 MSI: 105 IHC: 118	D-HPLC, PCR for direct automated sequencing, MLPA on samples from 10 patients who had tumours lacking at least one MMR protein expression and for which no previous mutation had been identified by sequencing.	Y	BAT25, BAT26, D2S123, D5S346, D17S250, BAT40, MYB, TGFRII, IGFIIR, BAX	Y	MLH1, MSH2, MSH6, PMS2

Study	Participants and selection	N	Ref standard	MSI	MSI Panel	IHC	IHC proteins
Single-gate studies recruiting populations at high-risk for Lynch syndrome							
Caldes, 2004[55]	HNPCC families selected through a clinic for familial cancer, selection process unclear	Recruited: 58 RS:58 MSI:58 IHC:58[c]	PCR, DGGE and sequencing. MSI-H cases that were negative for mutations in *MLH1*, *MSH2* and *MSH6* by DGGE and direct sequencing were analysed for genomic deletions in *MSH2* and *MLH1* by Southern blotting.	Y	BAT25, BAT26, D2S123, D5S346 and D17S250	Y	MLH1, MSH2, MSH6
Mueller, 2009[56]	'Suspected Lynch syndrome' participants who met Amsterdam criteria, modified Amsterdam criteria, were 'HNPCC-like' or met Bethesda criteria, selection process unclear	Recruited: 48[d] RS:48 MSI:48 IHC:48	Sequencing and MLPA	Y	5 and 10 panel markers, no further details provided	Y	MLH1, MSH2, MSH6, PMS2
Overbeek, 2007[57]	Families history that fulfilled one of the following criteria: 1) Amsterdam II criteria 2) Bethesda guidelines 3) a history very close to the Bethesda guidelines, selection process unclear	Recruited: 83 RS:83 MSI:43 IHC: Unclear	SSCP or DGGE and direct sequence analysis. MLPA for the detection of large deletions and duplications (confirmed by Southern blot analysis or with a specific PCR using primers flanking the deletion or one of the breakpoints of a duplicated region).	Y	BAT25, BAT26, D2S123, D5S346, D17S250 (BAT40 was also added to the standard set of markers but it is unclear for which participants)	Y	MLH1, MSH2, MSH6 PMS2
Poynter, 2008[a; 31]	Recruitment through high-risk clinics (clinic-based sample), selection process unclear[b]	Recruited: 172 RS: 152 MSI: 172 IHC: 157	For *MSH2* and *MLH1*: a combined approach of D-HPLC/direct sequencing and multiplex ligation dependent probe amplification (MLPA). For *MSH6*: Direct sequencing in cases with absent immunohistochemical staining of MSH6.	Y	BAT25, BAT26, D5S346, D17S250, BAT40, MYCL, ACTC, Dl 8S55, D1OS197, BAT34C4	Y	MLH1, MSH2, MSH6 PMS2

Study	Participants and selection	N	Ref standard	MSI	MSI Panel	IHC	IHC proteins
Shia, 2005[58]	Family history that fulfilled one of the following criteria: I) Amsterdam I or II criteria 2) a set of relaxed AC three or more colorectal cancers among the first and second-degree relatives of a family that we referred to as "HNPCC-like," and 3) Bethesda criteria, selection process unclear	Recruited: 83 RS:83 MSI: Unclear [e] IHC: Unclear [e]	D-HPLC, followed by direct sequencing for DNA fragments that displayed an abnormal chromatogram. Analysis for large deletions (multiplex PCR of short florescent fragments) only performed in MSI high tumours where a point mutation was not detected.	Y	BAT25, BAT26, D2S123, D17S250, BAT40, PAX6, MYCL1	Y	MLH1, MSH2, MSH6
Reference standard positive studies (recruiting populations with known mutation status)							
Hendriks, 2003[59]	Germline mutation in *MLH1*, *MSH2* or *MSH6*, selection process unclear	Recruited: 45 RS:45 MSI:33 IHC: 45	DGGE or Southern blotting	Y	BAT25, BAT26, D2S123, D5S346, D17S250, BAT40, MSH3 and MSH6	Y	MLH1, MSH2, MSH6
Okkels, 2012[60]	Germline mutation in *MSH6*, consecutive recruitment	Recruited: 56 RS:56 IHC:56	PCR and sequencing in sense and anti-sense directions, MLPA	N	N/A	Y	MLH1, MSH2, MSH6, PMS2 [f]

Notes: [a] Poynter et al. (2008) reports data from two distinct samples, a population-based sample and a high-risk sample; [b] Although Poynter et al. (2008) reports that 'some centres recruited all incident cases of CRC while others over- sampled cases with a family history or early age of onset' it is not clear whether this applies to the high-risk sample alone or in part to the high-risk sample ad in part to the population based sample; [c] In five cases IHC was not conducted for all proteins; [d] Number of participants recruited with a CRC tumour; [e] MSI data are available for 61 participants and IHC data for 64 participants, but it is unclear how many received the tests; [f] PMS2 not performed in all cases, data from MSH6 only included in this review

2.2.2.2 Single-gate studies recruiting high-risk populations

The five single-gate studies that were based on high-risk populations (Caldes, 2004; Mueller, 2009; Overbeek, 2007; the other sample reported in Poynter, 2008; Shia, 2005) all applied different criteria to select participants for inclusion.[31, 55-57] For two of these studies (Caldes, 2004; Poynter, 2008)[31, 55] the participant inclusion criteria were unclear, although for the study by Caldes et al. (2004) it was reported that participants were recruited from a familial cancer clinic and for the study by Poynter et al. (2008) it was reported that participants were recruited through high-risk clinics and that some clinics selected participants with a 'family history or early age of onset'. The remaining three single-gate studies appeared to include participants that were high-risk because of their family history (*Table 6*). These five studies varied in size with the largest study being Poynter et al. (2008), recruiting 172 participants and the smallest study being Mueller et al. (2009), recruiting 48 participants.[31, 56]

With regards to the population characteristics of these five studies, only one provided details on the participants' gender (Shia, 2005: 56.3% female; *Table 7*). The age of participants was reported in two of the five studies (Overbeek, 2007; Shia, 2005) with the age ranges reported as 29–51 years in Overbeek et al. (2007) and 23–78 years in Shia et al. (2005).[57, 58] Only one of the five studies (Shia 2005) reported the proportion of participants meeting AMS II criteria (38.2%) or RBG (8.2%) and the specific location of the CRC was only reported by Overbeek et al. (2007) and is given in *Table 7*.[57, 58] None of the studies provided details on the ethnicity of participants.

All five of the single-gate studies recruiting high-risk populations assessed both MSI and IHC.

2.2.2.3 Reference standard positive studies

There were two reference standard positive studies (i.e., studies that used a variation on a two-gate study design where only participants who were reference standard positives were recruited: Hendriks, 2003; Okkels, 2012).[59, 60] The study by Hendriks et al. (2003) recruited participants with a germline mutation in *MLH1*, *MSH2* or *MSH6* and assessed both MSI and IHC, whereas the study by Okkels et al. (2012) was focused on the identification of a germline mutation in *MSH6* and assessed only IHC (*Table 6*).[59, 60] It should be noted that although the study by Okkels et al. (2012) provides an assessment of the test accuracy of four proteins (MLH1, MSH2, MSH6, PMS2) and data are provided for all proteins combined, these data are not included in this review.[60] Instead the IHC results for MSH6 only are included.

Both of the reference standard positive studies including in this review are quite small, with Hendriks et al. (2003) recruiting 45 participants and Okkels et al. (2012) recruiting 56 participants.[59, 60]

The study by Okkels et al. (2012) did not provide details on the participants' age, gender, cancer location, or on the number of participants meeting AMS-II criteria, or RBG.[60] The study by Hendriks et al. (2003) reported a similar proportion of males and females (44% female), a participant age range of 23-90 years, and also reported the specific locations of the CRC (*Table 7*).[59] The proportion meeting AMS II criteria, or RBG, was not reported. Neither of the reference standard positive studies reported participants' ethnicity.

Table 7: Population characteristics of included studies

Author, year	Mean/median age in years	No. meeting AMS II/RBG criteria (%)	Gender, n (%)	Cancer location, n (%)
Single-gate studies recruiting population-based samples				
Barnetson, 2006[52]	Non-carrier 48.2 (±6.0) Carrier 42.7 (±7.7) *MLH1* 38.5 (±8.4) *MSH2* 43.8 (±6.1) *MSH6* 49.0 (±3.9)	AMS II 34 (4) RBG 555 (64)	Male 462 (53.1) Female 408 (46.9)	*Carrier* Rectum 7 (18.4) Sigmoid colon and rectosigmoid 7 (15.5) Descending colon 2 (5.3) Ascending colon/hepatic flexure 10 (26.3) Caecum 9 (23.7) Transverse colon 3 (7.9) *Non-carrier* Rectum 285 (35.2) Sigmoid colon and rectosigmoid 249 (30.7) Descending colon 37 (4.6) Splenic flexure 21 (2.6) Ascending colon/hepatic flexure 68 (8.4) Caecum 110 (13.6) Appendix 10 (1.2) Transverse colon 30 (3.7) Site not assessable 22 (2.6)

Limburg, 2011[53]	42.9 (±6.1)	AMS II 10 (5.1)	Male 91 (47) Female 104 (53)	NR
Poynter, 2008[a; 31]	NR	*Methylated* AMS II 6 (0.6)RBG 81 (7.6) *Unmethylated (loss of MLH1)* AMS II 10 (0.9) RBG 39 (3.7) *Unmethylated (loss of other MMR)* AMS II 17 (1.6) RBG 50 (4.7) *Unmethylated (no MMR loss)* AMS II 1 (0.1) RBG 20 (1.9)	*Methylated* Male 44 (4.1) Female 125 (11.8) *Unmethylated (loss of MLH1)* Male 25 (2.4) Female 26 (2.4) *Unmethylated (loss of other MMR)* Male 35 (3.3) Female 32 (3.0) *Unmethylated (no MMR loss)* Male 16 (1.5) Female 10 (0.9)	*Methylated* Rectum 4 (0.4) Left colon 9 (0.8) Right colon 155 (14.6) *Unmethylated (loss of MLH1)* Rectum 3 (0.3) Left colon 6 (0.6) Right colon 41 (3.9) *Unmethylated (loss of other MMR)* Rectum 7 (0.7) Left colon 12 (1.1) Right colon 45 (4.2) *Unmethylated (no MMR loss)* Rectum 15 (1.4) Left colon 8 (0.7) Right colon 40 (3.8)
Southey, 2005[54]	IHC 37.2 (range 24 to 42) MSI 37.2 (range 24 to 42) RS 37.1 (range 24 to 42)	AMS II 12 (9.2)	*IHC* Male 59 (45.0) Female 46 (35.1) *MSI* Male 59 (45.0) Female 46 (35.1) *Reference standard* Male 37 (28.2) Female 22 (16.8)	NR
Single-gate studies recruiting populations at high-risk for Lynch syndrome				
Caldes, 2004[55]	NR	NR	NR	NR
Mueller, 2009[56]	NR	NR	NR	NR

Overbeek, 2007[57]	40.7 (range 29 to 51)	NR	NR	Rectum 4 (4.8) Colon 2 (2.4) Splenic flexure 1 (1.2) Ascending colon 2 (2.4) Ileocaecum 1 (1.2)
Poynter, 2008[b; 31]	NR	NR	NR	NR
Shia, 2005[58]	Mean 50.5 Median 50 (range 23 to 78)	AMS II 42 (38.2) RBG 9 (8.2)	Male 48 (43.6) Female 62 (56.3)	NR

Reference standard positive studies (recruiting populations with known mutation status)				
Hendriks, 2003[59]	*Reference standard/IHC* *MLH1* 46 (range 28 to 90) *MSH2* 40 (range 23 to 61)[a] *MSH6* 62 (range 26 to 84) *MSI* *MLH1* 48 (range 29 to 90) *MSH2* 40 (range 23 to 61)[a] *MSH6* 62 (range 26 to 84)	NR	*Reference standard/IHC* Male 25 (56.0) Female 20 (44.0) *MSI* Male 16 (35.6) Female 18 (40.0)	*Reference standard/IHC* Rectum 1 (2.2) Colon 12 (2.7) Descending colon 1 (2.2) Sigmoid colon 4 (8.9) Splenic flexure 1 (2.2) (Duodenum 1 [2.2])[c] Ascending colon 3 (6.7) Caecum 12 (26.7) Hepatic flexure 2 (4.4) Transverse colon 8 (17.8) *MSI* Rectum 1 (2.2) Colon 4 (8.9) Descending colon 1 (2.2) Sigmoid colon 3 (6.7) Splenic flexure 1 (2.2) (Duodenum 1 [2.2])[c] Ascending colon 2 (4.4) Caecum 10 (22.2) Hepatic flexure 2 (4.4) Transverse colon 8 (17.8)
Okkels, 2012[60]	NR	NR	NR	NR

Notes: [a] Characteristics only for *MLH1* methylation in 313 MSI-H population-based cases with IHC data; [b] Data are not reported for the high-risk sample included in Poynter et al. (2008); [c] Study also includes one participant in the *MSH2* group with tumour site as duodenum

2.2.3 Summary of the reference standard in included studies

Three of the four studies that recruited a population-based sample (Barnetson, 2006; Poynter, 2008; Southey 2005) used a combination of direct sequencing and dHPLC analysis as the reference standard, followed by MLPA to detect large genomic alterations or deletions.[31, 52, 54] The study by Limburg et al. (2011) used direct sequencing but not dHPLC, followed by MLPA and Southern blot analysis.[53] All four studies investigated mutations in *MLH1*, *MSH2* and *MSH6*. However, in Poynter et al. (2008), direct sequencing was only used to detect *MSH6* mutations in cases with absent IHC staining of MSH6, and in Southey et al. (2005) mutations in *PMS2* were also investigated.[31, 54] In all four of these studies, large alterations or deletions were assessed in *MLH1* and *MSH2* but not *MSH6*, although in the study by Southey et al. (2005) this was only conducted for participants who had tumours lacking expression in at least one MMR protein and for which no previous mutation had been identified by sequencing.[54] In addition, it should be noted that in the population-based sample reported in Poynter et al. (2008), the reference standard was applied to all MSI-H and MSI-L participants and a random sample of MSS participants.[31] Further details are given in *Table 8*. It should also be noted that three of the four population-based studies (Poynter, 2008; Limburg, 2011; Barnetson, 2006) report on unclassified variants (i.e., mutations where the association with Lynch syndrome is unclear).[31, 52, 53] This can complicate the assessment of MSI in particular; mutations may be considered to be unclassified variants, with uncertain pathogenicity, because the variant may occur in cases with either MSI-H or MSS tumours. In this review, in primary analyses, unclassified variants have been counted as reference standard negatives. Secondary analyses have been conducted, as appropriate, where unclassified variants are considered to be reference standard positives. None of the population-based studies provided sufficient data on unclassified variants to be included in secondary analyses.

Of the five studies that reported data for high-risk populations,[31, 55-58] two (Caldes, 2004; Overbeek, 2007)[55, 57] used a combination of sequencing and DGGE, although in Overbeek et al. (2007), single-strand conformation polymorphism analysis was sometimes used instead of DGGE. The study by Caldes et al. (2004) followed this with Southern blot analysis whereas the study by Overbeek et al. (2007) used a combination of MLPA and Southern blot analysis to detect large deletions.[55, 57] As reported above, Poynter et al. (2008) used a combination of direct sequencing and dHPLC as the reference standard, followed by MLPA to detect large genomic alterations or deletions.[31] Similarly, Shia et al. (2005) used dHPLC analysis and direct sequencing, but used a procedure based on the multiplex PCR of short fluorescent fragments for the detection of large deletions.[58] The study by Mueller et al. (2009) provided limited details on the reference standard, although it was clearly specified that MLPA was used.[56]

All five of the single-gate studies based on high-risk populations investigated mutations in *MLH1*, *MSH2* and *MSH6*,[31, 55-58] although, as mentioned above, Poynter et al. (2008) only used direct sequencing to detect *MSH6* mutations in cases with absent IHC staining of MSH6.[31] In addition, Overbeek et al. (2007) investigated mutations in PMS2 and Mueller et al. (2009) investigated mutations in *PMS2* in cases that were mutation-negative for *MLH1*, *MSH2* and *MSH6*.[56, 57] For three of the five studies (Caldes, 2004; Poynter, 2008; Shia, 2005) large alterations or deletions were assessed in *MLH1* and *MSH2* but not *MSH6*.[31, 55, 58] It should be noted that in the studies by Caldes et al. (2004) and Shia et al. (2005) it was reported that large alterations or deletions were assessed only for MSI positive cases that

were mutation-negative.[55, 58] Mueller et al. (2009) does not clearly report which genes were assessed for large alterations or deletions.[56] For Overbeek et al. (2007) large deletions and duplications were assessed in *MLH1*, *MSH2*, *MSH6* and *PMS2*.[57] Two of these studies reporting data from high-risk populations report on unclassified variants (Caldes, 2004; Shia, 2005), but only one of these studies (Caldes, 2004) provides sufficient data to be included in secondary analyses (where unclassified variants are considered to be reference standard positives).[55, 58]

Both of the reference standard positive studies provide limited details on the reference standard (Hendriks, 2003; Okkels, 2012).[59, 60] The study by Okkels et al. (2012) used sequencing followed by MLPA and was focused only on *MSH6*.[60] The study by Hendriks et al. (2003) used DGGE followed by Southern blot analysis and assessed *MLH1*, *MSH2* and *MSH6*. Both studies recruited only reference standard positive participants and both studies recruited participants with, and report on, unclassified variants.[59] However, only Hendriks et al. (2003) provides sufficient data to be included in secondary analyses (where unclassified variants are considered to be reference standard positives).[59]

Table 8: Summary of the reference standard used in included studies

Author, year	Description	Participants receiving reference standard
Single-gate studies recruiting population-based samples		
Barnetson, 2006[52]	Germ-line DNA obtained from blood leukocytes analysed for *MLH1*, *MSH2*, and *MSH6* mutations. dHPLC analysis was used for *MSH2* and *MLH1*. Variants noted on chromatography were then sequenced. Mutations were confirmed by re-amplification of an independent sample of DNA and resequencing in both directions. *MLH1* and *MSH2* were assessed for deletions by MLPA, with products separated on a genetic analyser.	Mutational analysis and follow-up were complete in the total study population of 870.
Limburg, 2011[53]	DNA samples extracted from peripheral blood leukocytes received full mutation analyses of *MLH1*, *MSH2* and *MSH6*. DNA was amplified by PCR for each subject and directly sequenced in forward and reverse directions, using fluorescent dye-labelled sequencing primers: *MLH1*, *MSH2*, and *MSH6*. All potential genetic variants were independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination. Large rearrangement testing for *MLH1* and *MSH2* was performed by Southern blot analysis in conjunction with MLPA.	Germline *MLH1*, *MSH2* and *MSH6* sequencing data were obtained for 195 (100%), 195 (100%) and 189 (97%) subjects, respectively.
Poynter, 2008[a; 31]	Mutations in *MSH2* and *MLH1* were detected using a combined approach of dHPLC/direct sequencing and MLPA. Direct sequencing was used to detect *MSH6* mutations in cases with absent IHC staining of MSH6.	Population-based and clinic-based probands with CRC were tested for mutations in the MMR genes *MSH2*, *MLH1*, *MSH6*, and *PMS2*. MMR gene mutation testing for *MSH2* and *MLH1* was conducted for all clinic-based probands, all MSI-H or MSI-L population based probands, and in a random sample of 300 MSS population-based probands. MMR germline mutation status was available for 324/374 population-based MSI-H cases, 197/223 MSI-L cases, and 205/464 MSS cases.

Southey, 2005[54]	*MLH1*, *MSH2*, *MSH6*, and *PMS2* genes were screened for germline mutations using sequencing approaches or dHPLC. Confirmation of putative mutations was sought using an independent polymerase chain reaction for direct automated sequencing. MLPA was used to detect large genomic alterations in *MLH1* and *MSH2* on samples from 10 patients who had tumours lacking at least one MMR protein expression and for which no previous mutation had been identified by sequencing.	Ninety-two of 110 participants received germline mutation analysis. This included participants with one or more of the following characteristics: a family history that fulfilled the Amsterdam Criteria for hereditary nonpolyposis colorectal cancer (HNPCC); having a tumour that was high MSI, low MSI, or that lacked expression of at least one MMR protein; and presence in a random sample of 23 patients selected from those who had tumours that were MS stable and did not lack expression of any MMR protein.
Single-gate studies recruiting populations at high-risk for Lynch syndrome		
Caldes, 2004[55]	Genomic DNA was isolated from peripheral blood lymphocytes was analysed for *MLH1*, *MSH2* and *MSH6*. DNA was amplified using PCR and all amplicons were subjected to DGGE or cycle sequencing. The MSI-H cases that were negative for mutations were analysed for genomic deletions in *MLH1* and *MSH2* by Southern blotting.	Total population of 58 participants received germline mutation analysis.
Mueller, 2009[56]	Limited details. Deletion analysis was performed via MLPA.	Seventy-one CRC cases suspected to be Lynch syndrome cases were analysed for *MSH2*, *MLH1*, *MSH6*, and *PMS2* gene defects. Mutation-negative cases were screened for *MLH1* methylation and mutations in *PMS2*.
Overbeek, 2007[57]	Mutation analysis of *MLH1*, *PMS2*, *MSH2*, and *MSH6* was performed in DNA from peripheral blood lymphocytes by a combination of either single-strand conformation polymorphism analysis or DGGE and direct sequence analysis. For the detection of large deletions and duplications in *MLH1*, *MSH2*, *MSH6*, and *PMS2*, MLPA was used. All deletions and duplications were confirmed by Southern blot analysis or with a specific PCR.	Mutation analysis of germline DNA was performed as the first test in 83 families, who fulfilled clinical criteria for Lynch syndrome.

Poynter, 2008[a; 31]	Mutations in *MSH2* and *MLH1* were detected using a combined approach of dHPLC/direct sequencing and MLPA. Direct sequencing was used to detect *MSH6* mutations in cases with absent IHC staining of MSH6.	Population-based and clinic-based probands with CRC were tested for mutations in the MMR genes *MSH2*, *MLH1*, *MSH6*, and *PMS2*. MMR gene mutation testing for *MSH2* and *MLH1* was conducted for all clinic-based probands, all MSI-H or MSI-L population based probands, and in a random sample of 300 MSS population-based probands. MMR germline mutation status was available for 324/374 population-based MSI-H cases, 197/223 MSI-L cases, and 205/464 MSS cases.
Shia, 2005[58]	Each of the exons of *MLH1*, *MSH2* and *MSH6* was amplified by PCR, and heteroduplex analyses were performed using dHPLC. DNA fragments that displayed an abnormal chromatogram were sequenced directly. Cases with tumours that exhibited MSI but in which a point mutation was not detected were analysed for large deletions in *MLH1* and *MSH2* using a procedure based on the multiplex PCR of short fluorescent fragments.	Germline mutation was analysed in 83 participants with a carcinoma.
Reference standard positive studies (recruiting populations with known mutation status)		
Hendriks, 2003[59]	Limited details. Among the 35 HNPCC families with a known MMR defect, 27 different germline mutations were identified by DGGE or Southern blotting	All 45 patients (25 males and 20 females) had a known germline mutation in *MLH1*, *MSH2*, or *MSH6*.
Okkels, 2012[60]	Limited details. Standard sequencing of genomic DNA and MLPA	A total of 815 families were screened for *MSH6* mutations.

Notes: [a] Poynter et al. (2008) reports data from two distinct samples, a population-based sample and a high-risk sample

2.2.4 Quality appraisal of included studies

Quality appraisal was conducted, using Phase 3 of the QUADAS-2 tool, for all 11 data sets (all 10 studies, including both the population-based and high-risk samples reported in Poynter, 2008). Phase 3 of the QUADAS 2 tool contains four domains: patient selection, index tests, reference standard, and flow and timing. The quality of the included studies is discussed in the sections that follow according to these domains and is summarised in *Table 9*.

2.2.4.1 Patient selection

Four of the studies were rated as having a low risk of bias due to patient selection. Three of these were population-based single-gate studies (Barnetson, 2006; Limburg, 2011; Southey, 2005).[52-54] The other was a reference standard positive study (Okkels, 2012) from which only sensitivity estimates could be ascertained.[60] All four of these studies enrolled either a consecutive or random sample of participants and avoided inappropriate exclusions.

For the remaining seven studies (both samples reported in Poynter, 2008; Caldes, 2004; Mueller, 2009; Overbeek, 2007; Shia, 2005; Hendriks, 2003) it was unclear whether patient selection could have introduced bias.[31, 55-59] In all of these cases it was unclear whether inappropriate exclusions were avoided by enrolling a consecutive or random selection of participants.

For all studies included in the review, there were no concerns about whether or not the included participants matched the review question (*Table 9*).

2.2.4.2 Index tests

All of the studies included in the review of test accuracy evaluated IHC. With the exception of Limburg et al. (2011) and Okkels et al. (2012), all studies also assessed MSI.[53, 60] For both of these index tests, all studies were rated as unclear with regards to whether the conduct and interpretation of the test could have introduced bias; none of the studies clearly reported whether the thresholds used were pre-specified. In addition, none of the studies reported whether MSI results were interpreted without knowledge of the results of the reference standard. The study by Shia et al. (2005) reported that IHC results were interpreted without knowledge of the results of the reference standard, but for the remaining studies this was not reported.[58]

There were no concerns (in any of the studies) that the conduct or interpretation of either of the index tests was different from the review question (*Table 9*).

2.2.4.3 Reference standard

In all of the included studies the reference standard was assessed as likely to correctly classify the target condition. However, it should be noted that it has not been established that the reference standard is 100% sensitive, and that there is between-study variation in the reference standard (see *Section 2.2.3*, *page 82*). Nevertheless, because a genetic definition of Lynch syndrome is being used in this review (i.e., the reference standard is Lynch syndrome as indicated by a genetic mutation rather than, for example, Lynch syndrome defined by clinical criteria) and because the inclusion criteria for the reference standard has been set so that only studies using the best current methods, or other similarly appropriate methods, for detecting Lynch syndrome-based gene defects are included (see

Section 2.1.2.3) the assumption remains that any specific disagreements between the reference standard and the index test are assumed to result from incorrect classification by the index test. Indeed there were no concerns (in any of the studies) that the target condition, as defined by the reference standard, did not match the review question (*Table 9*).

Despite this, all of the included studies apart from Hendriks et al. (2003) were rated as unclear with regards to whether or not the conduct or interpretation of the reference standard could have introduced bias.[59] This was because only Hendriks et al. (2003) specified that the reference standard results were interpreted without knowledge of the results of the index test, with the rest of the studies not reporting this information. It is therefore unclear for these studies (Barnetson, 2006; Limburg, 2011; Southey, 2005; both sets of data in Poynter, 2008; Caldes, 2004; Mueller, 2009; Overbeek, 2007; Shia, 2005; Okkels, 2012) whether there was any prior knowledge that could have influenced the interpretation of the reference standard.[31, 52-58, 60]

2.2.4.4 Flow and timing

For all included studies, it was unclear whether the flow of participants through the study could have introduced bias. In most of the included studies all of the participants received a reference standard (Barnetson, 2006; Limburg, 2011; Caldes, 2004; Mueller, 2009; Overbeek, 2007;[52, 53, 55-57] the high-risk sample reported in Poynter, 2008; Shia, 2005; Hendriks, 2003; Okkels, 2012), although only one of these studies provided information to indicate that all of the participants received the same reference standard (Barnetson, 2006).[52] In five of these studies (Limburg, 2011; Mueller, 2009; Overbeek, 2007; Hendriks, 2003; Okkels, 2012)[53, 56, 57, 59, 60] it was not clear whether or not all participants received the same reference standard and in three of these studies (Caldes, 2004; high-risk sample reported in Poynter, 2008; Shia, 2005)[31, 55, 58] it was clear that not all participants received the same reference standard. Indeed, in Caldes et al. (2004) only the MSI-H cases that were negative for mutations were analysed for genomic deletions, by Southern blot analysis, in *MLH1* and *MSH2*.[55] Similarly, in Shia et al. (2005), only cases with tumours that exhibited MSI but in which a point mutation was not detected were analysed for large deletions in *MLH1* and *MSH2*.[58] In the high-risk sample in Poynter et al. (2008) direct sequencing was only used to detect *MSH6* mutations in cases with absent IHC staining of MSH6.[31] However, in these three cases it was not believed that this would constitute a high risk of bias; it is acceptable for large deletions to only be investigated when a mutation is not found (Caldes, 2004; Shia, 2005)[55, 58] and for the reference standard to be directed by IHC results (Poynter, 2008).[31]

In the other two samples (Southey, 2005; the population-based sample reported in Poynter, 2008)[31, 54] not all patients received the reference standard. In both of these samples this was because the reference standard was applied to a random sample of participants who were index test negative. In addition, in both of these samples (Southey, 2005; the population-based sample reported in Poynter, 2008),[31, 54] it was clear that not all of the patients who received the reference standard received the same reference standard; in the study by Southey et al. (2006) MLPA was used to detect large genomic alterations in *MLH1* and *MSH2* for cases with tumours lacking at least one MMR protein expression and for which no previous mutation had been identified by sequencing, and as with the high-risk sample in Poynter et al. (2008), in the population-based sample reported in Poynter et al. (2008), direct sequencing was only used to detect *MSH6* mutations in cases with absent IHC staining of

MSH6. As discussed above, these variations in study design would not be thought to constitute a high risk of bias.

None of the studies included in the test accuracy review clearly specified the interval between the index test(s) and the reference standard. However, as results on both of the index tests and on the reference standard would be expected to be stable over time, this information is of little importance in itself; variations in timing between the index tests and the reference standard would not lead to a high risk of bias.

With regards to missing data, it was clear that in most of the studies some of the participants were excluded from the analysis (Barnetson, 2006; Limburg, 2011; Southey, 2005; both sets of data in Poynter, 2008; Caldes, 2004; Hendriks, 2003; Okkels, 2012), although details regarding the characteristics of these participants were not provided.[31, 52-55, 59, 60] However the study by Mueller et al. (2009) clearly specifies that data are analysed from all participants who received tests.[56] In the studies by Overbeek et al. (2007) and Shia et al. (2005) it was unclear whether or not all tested participants were analysed.[57, 58]

2.2.4.5 Quality appraisal summary

Overall, there was no evidence found to indicate that any of the included studies were at high-risk of bias. Of course, the single gate studies based on a high-risk population (Caldes, 2004; Mueller, 2009; Overbeek, 2007; high-risk sample reported in Poynter, 2008; Shia, 2005) would necessarily confer a risk of bias (i.e., if the results were used to make assumptions about the general population with CRC).[31, 55-58] Indeed, when studies recruit only from high-risk populations this obviously would lead to biased estimates of PPV, NPV and yield. It would also possibly lead to biased estimates of sensitivity and specificity due to spectrum bias,[61] but in a previous review this did not appear to lead to significant bias in estimates of sensitivity.[39] As a result, this was dealt with in this review by pre-specifying that single-gate studies based on high-risk populations would only be used to estimate sensitivity. Ordinarily, two-gate studies would also be at risk of inflating diagnostic accuracy.[62, 63] However, the two studies included in this review that were not single-gate studies, were not in fact two-gate studies but reference standard positive studies (Hendriks, 2003; Okkels, 2012); no reference standard negatives were included in these two studies, so an unbiased estimate of sensitivity could be made.[59, 60]

It is important to note that an absence of evidence to suggest that the included studies were at high risk of bias does not suggest that the studies were at low risk of bias. In fact, for all studies it was unclear whether the index tests, or the flow and timing of the study, would have introduced bias. Similarly, in all but one study (Hendriks, 2003)[59] it was unclear whether the conduct of the reference standard would have introduced bias, and only four studies provided sufficient information to establish that the selection of participants was unlikely to have introduced bias (Barnetson, 2006; Limburg, 2011; Southey, 2005; Okkels, 2012).[52-54, 60]

Table 9: Quality appraisal of included studies based upon Phase 3 of QUADAS 2

Domain	Item	Population-based				High-risk, single-gate					Other	
		Barnetson, 2006[52]	Limburg, 2011[53]	Southey, 2005[54]	Poynter, 2008[a; 31]	Caldes, 2004[55]	Mueller, 2009[56]	Overbeek, 2007[57]	Poynter, 2008[31]	Shia, 2005[58]	Hendriks, 2003[59]	Okkels, 2012[60]
Patient selection	Was a consecutive or random sample of patients enrolled?	Y	Y	Y	U	U	U	U	U	U	U	Y
	Was a case-control design avoided?	Y	Y	Y	Y	Y	Y	Y	Y	Y	N[b]	N[b]
	Did the study avoid inappropriate exclusions?	Y	Y	Y	U	Y	U	U	U	U	Y	Y
	Could the selection of patients have introduced bias?	**L**	**L**	**L**	**U**	**U**	**U**	**U**	**U**	**U**	**U**[c]	**L**[d]
	Is there concern that the included patients do not match the review question?	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
Index test (MSI)	Were the index test results interpreted without knowledge of the results of the reference standard?	U		U	U	U	U	U	U	U	U	
	If a threshold was used, was it pre-specified?	U		U	U	U	U	U	U	U	U	
	Could the conduct or interpretation of the index test have introduced bias?	**U**		**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	
	Is there concern that the index test, its conduct, or interpretation differ from the review question?	**L**		**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	
Index test (IHC)	Were the index test results interpreted without knowledge of the results of the reference standard?	U	U	U	U	U	U	U	U	Y	U	U
	If a threshold was used, was it pre-specified?	U	U	U	U	U	U	U	U	U	U	U
	Could the conduct or interpretation of the index test have introduced bias?	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**
	Is there concern that the index test, its conduct, or interpretation differ from the review question?	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
Reference	Is the reference standard likely to correctly classify the target condition?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Domain	Item	Population-based				High-risk, single-gate					Other	
		Barnetson, 2006[52]	Limburg, 2011[53]	Southey, 2005[54]	Poynter, 2008[a; 31]	Caldes, 2004[55]	Mueller, 2009[56]	Overbeek, 2007[57]	Poynter, 2008[31]	Shia, 2005[58]	Hendriks, 2003[59]	Okkels, 2012[60]
standard	Were the reference standard results interpreted without knowledge of the results of the index test?	U	U	U	U	U	U	U	U	U	Y	U
	Could the reference standard, its conduct, or its interpretation have introduced bias?	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**L**	**U**
	Is there concern that the target condition as defined by the reference standard does not match the review question?	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**	**L**
Flow and timing	Was there an appropriate interval between index test(s) and reference standard?	U	U	U	U	U	U	U	U	U	U	U
	Did all patients receive a reference standard?	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y
	Did patients receive the same reference standard?	Y	U	N	N	N	U	U	N	N	U	U
	Were all patients included in the analysis?	N	N	N	N	N	Y	U	N	U	N	N
	Could the patient flow have introduced bias?	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**	**U**

Notes: [a] Poynter et al. (2008) was assessed twice because data were reported for both a population-based sample and a high-risk sample; [b] A case-control design was only avoided because there was no control group (half a case control study); [c] An unbiased estimate of sensitivity (but not specificity) can be ascertained from this study design, however an unclear rating is given because it is not clear if a consecutive or random sample was recruited; [d] An unbiased estimate of sensitivity (but not specificity) can be ascertained from this study design

2.2.5 Assessment of test accuracy

The index tests included in this review (MSI and IHC) are highly susceptible to spectrum effects in populations that have been selected due to clinical characteristics. Indeed, preselecting participants in this way will result in a population that differs from an unselected population, in terms of the clinical predictor, in a non-random way. Thus, the sensitivity and specificity estimates would likely be different among unselected CRC populations compared to CRC populations selected due to age, or due to characteristics which make them high-risk for Lynch syndrome (such as selection due to a family history of Lynch syndrome, or due to meeting clinical criteria for defining Lynch syndrome). In particular, increased presence of MMR mutation carriers in a population would change the apparent sensitivity and specificity of the index tests.[52] However, a previous review did not find that this issue led to significant bias in estimates of sensitivity.[39]

Due to this, the studies included in this review have been grouped by population, and results are presented accordingly. In addition, studies recruiting high-risk populations have only been used to estimate sensitivity. As previously described, four of the samples included in this review can be described as population-based samples, although only one recruited an unselected CRC population (Poynter, 2008),[31] with the other three recruiting age-limited populations (Barnetson, 2006; Limburg, 2011; Southey, 2005)[52-54] for which some spectrum bias may be expected. The remaining seven samples included in this review are all high-risk; five of the remaining studies had a single-gate design and recruited high-risk participants (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005)[31, 55-58] with the other two studies only recruiting participants who were reference standard positives (Hendriks, 2003; Okkels, 2012).[59, 60] For the five single-gate studies based on high-risk populations only sensitivity will be reported, even if data are available for other outcomes. For the two studies based on participants who were reference standard positives, only sensitivity is estimable from the data reported.

It is important to mention that none of the studies included in this review made a direct comparison between MSI and IHC. As such, results are reported separately for these tests.

2.2.5.1 Assessment of test accuracy for MSI

MSI was assessed in eight of the ten studies (nine of the eleven samples) included in the review of test accuracy: three of the four population-based samples (Barnetson, 2006; Southey, 2005; Poynter, 2008),[31, 52, 54] all five high-risk samples (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005),[31, 55-58] and one of the reference standard positive studies (Hendriks, 2003).[59]

2.2.5.1.1 MSI testing methods

A summary of the MSI testing methods used in these studies is provided in *Table 10*. It is evident that a variety of between-study differences exist in the MSI testing procedures used. In addition, differences between studies in MSI testing methods were not always clear because methods were not always reported in sufficient detail. For example, three of the eight studies assessing MSI (Poynter, 2008; Mueller, 2009; Overbeek, 2007) did not report microdissection techniques (microdissection assists in assuring that malignant tissue that does not contain DNA from surrounding, healthy colonic tissue is analysed).[31, 56, 57] A further two studies only reported very limited details about microdissection (Shia, 2005; Hendriks, 2003).[58, 59] Amongst the three studies that did report details regarding microdissection, there

was some variation in the technique used (Barnetson, 2006; Southey, 2005; Caldes, 2004; see *Table 10* for details).[52, 54, 55] The panel of markers used also differs between studies (*Table 10*). None of the population-based studies assessed the same panel of markers. Two of the three population-based samples (Barnetson, 2006; Southey 2005) included an assessment of the Bethesda panel of markers (BAT25, BAT26, D2S123, D5S346, D17S250), but Southey et al. (2005) also assessed five additional markers (BAT40, MYB, TGFRII, IGFIIR, and BAX).[52, 54] The other population-based study (the population-based sample included in Poynter, 2008) did not assess D2S123, even though the other Bethesda panel markers were included.[31] This study also assessed six additional markers (BAT40, MYCL, ACTC, Dl 8S55, D1OS197, and BAT34C4). The same panel of markers was used for the single-gate, high-risk sample included in Poynter et al. (2008).[31] Of the other four single-gate studies based on high-risk populations, three included the Bethesda panel of markers (Caldes, 2004; Mueller, 2009; Overbeek, 2007).[55-57] However, Overbeek et al. (2007) also assessed BAT40 for some participants (it is unclear which ones).[57] Also, Mueller et al. (2009) use a ten-marker panel (BAT26, BAT4O, Mfdl5, D2S123, APC, BAT25, D10S197, D18S58, D18S69, and MYCLJ) as well as a five-marker panel (the Bethesda panel), but it is unclear which participants received which panel of markers and which panel of markers the reported data are based upon.[56] The remaining single-gate study Shia et al. (2005) did not assess D5S346, but did assess the other Bethesda panel of markers, as well as BAT40, PAX6, and MYCL1.[58] It appears, therefore, that none of the single-gate studies based on high-risk samples used the same panel of markers (although this is unclear in the case of Mueller, 2009).[56] The reference standard positive study that assessed MSI included a different panel of markers as well: the Bethesda panel, plus BAT40, MSH3 and MSH6.

The eight studies that assessed MSI also varied in the way in which MSI was categorised (as a bimodal or trimodal distribution) and in the thresholds used to define these categories. Indeed, of the studies included in this review, five (Barnetson, 2006; Southey, 2005; Poynter, 2008; Mueller, 2009; Hendriks, 2003)[31, 52, 54, 56, 59] define tumours as MSI-H, MSI-L or MSS, also known as a trimodal distribution, two (Overbeek, 2007; Shia, 2005)[57, 58] define tumours as MSI positive or negative, also known as a bimodal distribution, and one (Caldes, 2004)[55] uses a bimodal distribution but defines tumours as either MSI-H or MSS.

It is also unsurprising that the thresholds used to categorise tumours (as MSI-H, MSI-L, or MSS or as MSI positive or negative) differ between studies; the distinction between these categories is dependent on both the type and number of microsatellites analysed, and as discussed above, the studies included in this review use various different panels of makers.[64] Indeed, with regards to the trimodal distribution of MSI, many groups define MSI-H tumours as those with more than 30–40% unstable markers, MSI-L as instability lower than this threshold, and MSS as no instability.[64] Of the five studies that use the MSI-H, MSI-L and MSS categories (trimodal distribution), the thresholds used to categorise the tumours vary greatly, with one of these studies using the commonly used threshold of more than 30% of unstable markers to define MSI-H tumours (Poynter, 2008), three studies using differing numbers of unstable markers to define MSI-H tumours, and one study (Mueller, 2009) not providing details on the thresholds used to categorise the tumours as MSI-H, MSI-L and MSS (see *Table 10* for details).[31, 56] Of the two studies that defined tumours as positive or negative (bimodal distribution), one reported using a threshold of more than 30% of unstable markers to define MSI positive tumours (Shia, 2005) and the other defined MSI positive tumours as those with more than two unstable Bethesda panel markers (Overbeek, 2007).[57, 58] The study by Caldes et al. (2004) defined MSI-H tumours as those with two or more

unstable Bethesda markers (or one marker in the case of BAT26), and MSS tumours as those showing no instability. It is not clear how cases with only one unstable marker (other than BAT26) were categorised, but in any case data are only presented for tumours that were categorised as MSS and MSI-H.[55]

It has been asserted by Pawlik et al. (2004)[64] that a bimodal distribution of MSI (as used in Shia, 2005 and Overbeek, 2007)[57, 58] may be more useful than a trimodal distribution of MSI (as used in Barnetson, 2006; Southey, 2005; Poynter, 2008; Caldes, 2004; Mueller, 2009; Hendriks, 2003).[31, 52, 54-56, 59] Indeed, in studies using a trimodal distribution, MSI-L can be considered as either positive or negative and although MSI-L tumours may behave more similarly to MSS tumours in clinical and prognostic terms, the significance of MSI-L is still uncertain, and would vary according to the particular markers used.[64] Clearly, a range of markers are used in the studies included in this review. Thus, for studies using a trimodal distribution of MSI, and where data are available, the sensitivity and specificity of the MSI test has been estimated separately with MSI-L values considered as index test positives and with MSI-L values considered as index test negatives.

Table 10: Details of MSI testing in included studies

Study	Microdissection	MSI markers	Threshold		
			MSI-H	MSI-L	MSS
Population-based single-gate studies					
Barnetson, 2006[52]	10µm tumour sections; microdissection performed on purified tumour DNA, and control DNA from blood or normal tissue in the section	BAT25, BAT26, D2S123, D5S346, D17S250	>1 marker	1 marker	0 markers
Southey, 2005[54]	5µm tumour sections; microdissection performed on invasive tumour cells from paraffin-embedded archival tumour tissue stained with 1% methyl-green, and normal cells from colonic or lymph node tissue/DNA extracted from peripheral blood lymphocytes	BAT25, BAT26, D2S123, D5S346, D17S250, BAT40, MYB, TGFRII, IGFIIR, BAX	>5 markers	2-5 markers	<2 markers
Poynter, 2008[a, b; 31]	Not reported	BAT25, BAT26, D5S346, D17S250, BAT40, MYCL, ACTC, Dl 8S55, D1OS197, BAT34C4	≥30%	>0% and <30%	0%
Single-gate studies recruiting 'high risk' samples					
Caldes, 2004[55]	10µm tumour sections; microdissection performed on H&E stained slides with demarked areas containing cancer cells, and corresponding areas on unmarked slides	BAT25, BAT26, D2S123, D5S346, D17S250	>1 marker, or 1 marker if BAT26	Not used	0 markers
Mueller, 2009[56]	Not reported	5 and 10 panel markers [c]	Not reported [d]		
Overbeek, 2007[57]	Not reported	BAT25, BAT26, D2S123, D5S346, D17S250, BAT40 [e]	Tumours categorised as positive (>2 Bethesda markers) or negative		
Poynter, 2008[a, b; 31]	Not reported	BAT25, BAT26, D5S346, D17S250, BAT40, MYCL, ACTC, Dl 8S55, D1OS197, BAT34C4	≥30%	>0% and <30%	0%
Shia, 2005[b; 58]	Microdissection performed on DNA from paraffin-embedded tissue blocks. No further details reported	BAT25, BAT26, D2S123, D17S250, BAT40, PAX6, MYCL1	Tumours categorised as positive (≥30%) or negative		

Study	Microdissection	MSI markers	Threshold		
			MSI-H	MSI-L	MSS
Reference standard positive study					
Hendriks, 2003[59]	Microdissection not specifically reported, paired tumour and normal tissue DNA samples were used	BAT25, BAT26, D2S123, D5S346, D17S250, BAT40, MSH3 and MSH6	>1 Bethesda markers	1 Bethesda marker	0 Bethesda markers

Notes: [a] Poynter et al. (2008) includes both a population-based sample and a high-risk sample; [b] Poynter et al. (2008) and Shia et al. (2005) give thresholds as proportions of successfully typed loci rather than as number of markers; [c] References are provided to Boland et al. (1998)[65] who recommend the use of BAT25, BAT26, D2S123, D5S346, and D17S250 and Dietmaier et al. (1997)[66] who recommend the use of BAT26, BAT4O, Mfdl5, D2S123, APC, BAT25, D10S197, D18S58, D18S69, and MYCLJ; [d] Defined as MSI-H, MSI-L and MSS, but thresholds are not described; [e] BAT40 was added to the standard set of markers but it is unclear for which participants

2.2.5.1.2 MSI-H versus MSS+MSI-L

Three population-based samples (Poynter, 2008; Barnetson, 2006; Southey, 2005),[31, 52, 54] two high-risk single-gate study samples (Mueller, 2009; Poynter, 2008),[31, 56] and one reference standard positive study sample (Hendriks, 2003)[59] provided test accuracy data for MSI where MSI-L cases were considered to be index test negatives. In the other three studies assessing MSI (Overbeek, 2007; Shia, 2005; Caldes, 2004),[55, 57, 58] MSI was already categorised as a bimodal distribution (MSI positive or negative for Overbeek, 2007 and Shia, 2005; MSI-H or MSS for Caldes, 2004). For ease of reference, the results from these three studies are included here and again in the section reporting data where MSI-L tumours are considered to be positive.

Sensitivity and specificity estimates

Sensitivity was calculated for all studies that provided data where MSI-L was considered to be index test negative (as well as the studies categorising MSI as a bimodal distribution), whereas specificity was calculated only for the three population-based studies (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] These sensitivity and specificity estimates are reported in *Table 11*.

Only one study included a population that was unselected (not limited by age or risk) and the data from this study (Poynter, 2008) produced a sensitivity estimate of 100% (95% CI 93.9, 100.0) and specificity of 61.1 (95% CI 57.0, 65.1).[31] It should be noted that in this sample and in the data reported for this study in *Table 11*, unclassified variants were considered to be reference standard negatives. Two of the studies that reported data where MSI-L was considered to denote an index test negative were based on age-limited populations (Barnetson, 2006; Southey, 2005).[52, 54] The study by Barnetson et al. (2006) included unclassified variants as reference standard negatives and is reported in this section as such.[52] In these two studies, sensitivity and specificity estimates were fairly similar, despite the fact that different panels of markers were used alongside different thresholds to categorise the tumours (*Table 10*): sensitivity was 66.7% (95% CI 47.2, 82.7) in Barnetson et al. (2006) and 72.2% (95% CI 46.5, 90.3) in Southey et al. (2005) and specificity was 92.5% (95% CI 89.1, 95.2) in Barnetson et al. (2006) and 87.8% (95% CI 73.8, 95.9) in Southey et al. (2005) (*Table 11*).[52, 54]

Table 11: Sensitivity and specificity for MSI (MSI-H versus MSI-L or MSS)

Author, year	Sensitivity (%)	Specificity (%)
Single-gate, population-based samples		
Poynter, 2008[a; 31]	100.0 (93.9, 100.0)	61.1 (57.0, 65.1)
Barnetson, 2006[52]	66.7 (47.2, 82.7)	92.5 (89.1, 95.2)
Southey, 2005[54]	72.2 (46.5, 90.3)	87.8 (73.8, 95.9)
Single-gate, high-risk samples		
Caldes, 2004[b; 55]	79.4 (62.1, 91.3)	—
Mueller, 2009[56]	91.3 (72.0, 98.9)	—
Overbeek, 2007[b; 57]	90.0 (59.6, 98.2)	—
Poynter, 2008[31]	86.8 (71.9, 95.6)	—
Shia, 2005[b 58]	100.0 (85.8, 100.0)	—
Reference standard positive study		
Hendriks, 2003[59]	88.0 (68.8, 97.5)	—

Notes: [a] Population based sample; [b] MSI-L not defined; [c] clinic based sample

For the five single-gate, high-risk samples presented in *Table 11* (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005),[31, 55-58] sensitivity estimates ranged from 79.4% (95% CI 62.1, 91.3) in Caldes et al. (2004) to 100.0% (95% CI 85.8, 100.0) in Shia et al. (2005). Two of these high-risk, single gate studies, mention unclassified variants (Caldes, 2004; Shia, 2005) and these are counted as reference standard negatives in these analyses (*Table 11*).[55, 58] Between-study variation in sensitivity estimates may be due to a variety of factors including differences in the panel of markers used, and in the MSI thresholds used to denote cases, as well as differences in the reference standard. Nevertheless, all of these sensitivity estimates were >79%. It should be noted that Caldes et al. (2004) report that the sensitivity of MSI-H in predicting a pathogenic mutation was 96% (95% CI 90, 100) in contrast to our calculation of 79.4% (95% CI 62.1, 91.3).[55] This is because Caldes et al. (2004) excluded five cases from their MSI analyses which did not also have IHC data, whereas these cases were included in our calculations. The data from the reference standard positive study that assessed MSI (Hendriks, 2003) was also used to generate a sensitivity estimate; when MSI-L cases were considered to be index test negatives, sensitivity was 88.0% (95% CI 68.8, 97.5).[59]

Due to a potential for spectrum bias, it would be expected that studies recruiting high-risk populations would result in higher sensitivity estimates than those estimated from population-based studies. However, as discussed in a systematic review by Palomaki et al. (2009), we did not find great differences between the sensitivity estimates in the population-based studies and the high-risk studies.[39] Indeed, although two of the three population-based studies produced the lowest sensitivity estimates (Barnetson, 2006; Southey, 2005), the other population-based study produced a sensitivity estimate of 100% (95% CI 93.9, 100.0).[52, 54] In fact, the two population-based studies with lower sensitivity estimates would, in theory, be more likely to be subject to some spectrum bias than the study by Poynter et al. (2008) because they are based on age-limited populations.[31] This highlights how comparison between the studies included in this review may not be meaningful; other factors such as the particular MSI methods, panel of markers and thresholds, as well as methods used to conduct the reference standard varied between studies.

For three of the five single-gate high risk samples (Poynter, 2008; Mueller, 2009; Overbeek, 2007) data regarding methylation are reported.[31, 56, 57] In Poynter et al. (2008) it is reported that the prevalence of *MLH1* methylation in MSI-H tumours was much lower in the high-risk sample than in the population-based sample at 13% (versus 60% in the population-based sample; $P < 0.0001$) with none at all in the MSI-L or MSS tumours from the high-risk sample.[31] The authors suggest this may be due to the higher frequency of clinic-based MSI-H cases with a MMR germline mutation and because the MSI-H cases in the population based series were diagnosed at an older age than the clinic-based series (median age 63 years, range 22–75 years versus median age 44 years, range 19–77 years, respectively; Poynter, 2008).[31] Despite recruiting a high risk population, Mueller et al. (2009) report that of the seven MSI-H cases where no mutation was identified by the reference standard, four had somatic silencing of *MLH1* and were likely to be sporadic cases.[56] Overbeek et al. (2007) did not test all participants relevant to this review for *MLH1* promoter methylation.[57] However, of those tested, none were positive.

Other test accuracy estimates

The other test accuracy outcomes included in this review were likelihood ratios (LR+ and LR−), PPV and NPV, accuracy or concordance with the reference standard, diagnostic yield, and test failure rates. The latter three outcomes (accuracy or concordance with the reference standard, diagnostic yield and test failure rates were not reported in any of the included studies).The other four outcomes (LR+, LR−, PPV and NPV) were calculated for the three population-based studies reporting MSI data (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] These outcomes are reported in *Table 12* and are based on data where MSI-L was assumed to be a negative index test result and the unclassified variants reported in Barnetson et al. (2006) and Poynter et al. (2008) were assumed to be negative reference standard results.[31, 52]

Table 12: Likelihood ratios and predictive values for MSI (MSI-H versus MSI-L or MSS)

Author, year	LR+	LR−	PPV (%)	NPV (%)
Single-gate, population-based samples				
Poynter, 2008[a; 31]	2.57 (2.32, 2.85)	0.00 (NE)[b]	20.8 (16.2, 26.0)	100.0 (99.0,100.0)
Barnetson, 2006[52]	8.94 (5.54, 14.20)	0.36 (0.21, 0.60)	45.5 (30.4, 61.2)	96.8 (94.1, 98.4)
Southey, 2005[54]	5.92 (2.48, 14.10)	0.32 (0.15, 0.67)	72.2 (46.5, 90.3)	87.8 (73.8, 95.9)

Notes: [a] Population based sample; [b] Not estimable

As can be seen in *Table 12*, results were fairly consistent amongst the three studies (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] LR+ ranged from 2.57 (95% CI 2.32, 2.85) for Poynter (2008) to 8.94 (95% CI 5.54, 14.20) for Barnetson et al. (2006).[31, 52] LR− could only be estimated for two of the studies (Barnetson, 2006; Southey, 2005)[52, 54] because there were no false negative MSI results in Poynter (2008).[31] LR− was similar in both studies (Barnetson, 2006; Southey, 2005; *Table 12*).[52, 54] PPV (the probability of someone with a positive result actually having Lynch syndrome as defined by the reference standard) varied a lot more between these studies with the lowest estimate coming from Poynter et al. (2008) at 20.8% (95% CI 16.2, 26.0) and the highest from Southey et al. (2005) at 72.2% (95% CI 46.5, 90.3).[31, 54] It should be noted that the confidence intervals generated from Southey et al. (2005) are relatively wide, which reflects the smaller sample size of 59, as opposed to 638 samples for Poynter et al. (2008) and 352 samples for

Barnetson et al. (2006).[31, 52, 54] NPV (the probability of someone with a negative test result actually not having Lynch syndrome as defined by the reference standard) was consistent across these studies: all estimates were >87%.

Secondary analyses (unclassified variants as index test positives)

Secondary analyses were conducted where unclassified variants were considered to indicate a positive reference standard test result. It was not possible to include all studies reporting MSI data in these analyses (because sufficient data were not reported). Five studies (the population-based sample in Poynter, 2008; Barnetson, 2006; Caldes, 2004; Shia, 2005; Hendriks, 2003) reported unclassified variants in their assessment of MSI.[31, 52, 55, 58, 59] Of these five studies, only two (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary analyses.[55, 59] However, because Caldes et al. (2004) was based on a high-risk population and Hendriks et al. (2003) is a reference standard positive study, only sensitivity estimates were made.[55, 59]

Thus, when unclassified variants were considered to be reference standard positives, and MSI-L was considered to be an index test negative, Caldes et al. (2004) reported sensitivity as 81.6% (95% CI 65.7, 92.3) and Hendriks et al. (2003) reported sensitivity as 84.8% (95% CI 69.0, 93.3).[55, 59] These results were similar to those obtained when unclassified variants were considered to be negative (79.4% [95% CI 62.1, 91.3] for Caldes, 2004; 88.8% [95% CI 68.8, 97.5] for Hendriks, 2003; *Table 11*).[55, 59]

2.2.5.1.3 MSI-H and MSI-L versus MSS

When MSI-L tumours were considered to be index test positives, data were available from all three population-based samples that assessed MSI (Poynter, 2008; Barnetson, 2006; Southey, 2005),[31, 52, 54] two high-risk single-gate study samples (Mueller, 2009; Poynter, 2008),[31, 56] and one reference standard positive study sample (Hendriks, 2003).[59] As previously discussed, the other three studies assessing MSI (Overbeek, 2007; Shia, 2005; Caldes, 2004)[55, 57, 58] already categorise MSI as a bimodal distribution (MSI positive or negative for Overbeek, 2007 and Shia, 2005; MSI-H or MSS for Caldes, 2004). The results from these three studies are included here as well as in *Section 2.2.5.1.2* above.

Sensitivity and specificity estimates

Sensitivity was calculated for all studies that provided data where MSI-L was considered to be index test positive (as well as the studies categorising MSI as a bimodal distribution). Specificity was calculated only for the three population-based study samples (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] These sensitivity and specificity estimates are reported in *Table 13*. For the five studies that mention assessing unclassified variants in addition to pathogenic mutations (the population-based sample in Poynter, 2008; Barnetson, 2006; Caldes, 2004; Shia, 2005; Hendriks, 2003), the unclassified variants were considered to be reference standard negatives.[31, 52, 55, 58, 59]

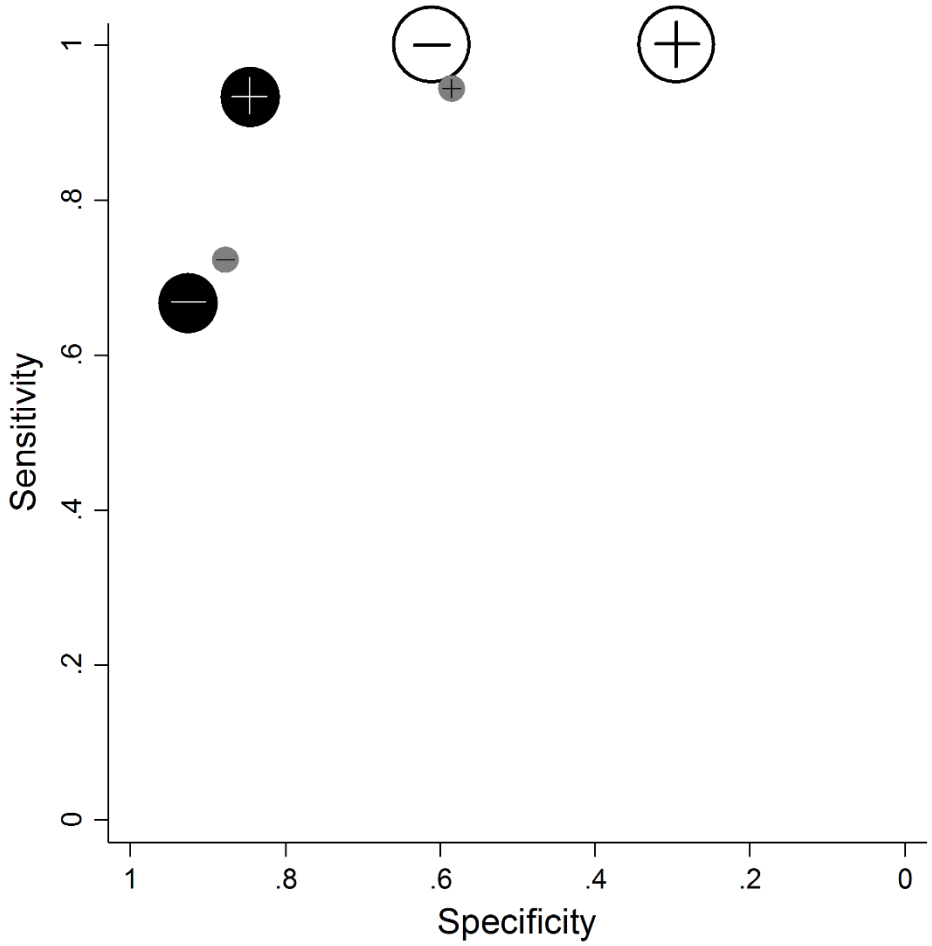
Table 13: Sensitivity and specificity for MSI (MSI-H or MSI-L versus MSS)

Author, year	Sensitivity (%)	Specificity (%)
Population-based single-gate samples		
Poynter, 2008[a; 31]	100.0 (93.9, 100.0)	29.5 (25.8, 33.4)
Barnetson, 2006[52]	93.3 (77.9, 99.2)	84.5 (80.0, 88.2)
Southey, 2005[54]	94.4 (72.7, 99.9)	58.5 (42.1, 73.7)
High-risk single-gate samples		
Caldes, 2004[b; 55]	79.4 (62.1, 91.3)	—
Mueller, 2009[56]	93.1 (77.2, 99.2)	—
Overbeek, 2007[b; 57]	90.0 (59.6, 98.2)	—
Poynter, 2008[31]	94.7 (82.3, 99.4)	—
Shia, 2005[b; 58]	100.0 (85.8, 100.0)	—
Reference standard positive study sample		
Hendriks, 2003[59]	92.0 (74.0, 99.0)	—

Notes: [a] Population based sample; [b] MSI-L not defined; [c] clinic based sample

The data from the study that included an unselected CRC population (Poynter, 2008) produced a sensitivity estimate of 100.0% (95% CI 93.9, 100.0) and therefore, sensitivity was unchanged from when MSI-L cases were considered to be index test negatives (see *Section 2.2.5.1.2* above).[31] However, specificity was much lower, at 29.5% (95% CI 25.8, 33.4), compared with 61.1 (95% CI 57.0, 65.1) when MSI-L was considered to be a negative index test result, reflecting a large increase in false positive results. The two studies based on age-limited populations that reported data where MSI-L was considered to be an index test positive result (Barnetson, 2006; Southey, 2005) reported sensitivities of 93.3% (95% CI 77.9, 99.2) and 94.4% (95% CI 72.7, 99.9) respectively.[52, 54] These sensitivities were higher than those estimated when MSI-L was considered to be an index test negative result where sensitivity was estimated at 66.7% (95% CI 47.2, 82.7) for Barnetson et al. (2006) and 72.2% (95% CI 46.5, 90.3) for Southey et al. (2005).[52, 54] As would be expected in a trade-off between sensitivity and specificity, specificities were reduced for these two studies when MSI-L cases were considered to be index test positives. However, for Barnetson et al. (2006) this reduction was small with specificity estimated as 84.5% (95% CI 80.0, 88.2) when MSI-L was considered to be a positive index test result (*Table 13*) and as 92.5% (95% CI 89.1, 95.2) when MSI-L was considered to be a negative index test result (*Table 11*, *Section 2.2.5.1.2*).[52] For Southey et al. (2005), specificity was estimated as 58.5% (95% CI 42.1, 73.7) when MSI-L was considered to be a positive index test result (*Table 13*) and as 87.8% (95% CI 73.8, 95.9) when MSI-L was considered to be a negative index test result (*Table 13*, *Section 2.2.5.1.2*).[54]To further demonstrate the difference in test performance between MSI-L as a positive test result and MSI-L as a negative test result, sensitivities and specificities were graphically summarised using a receiver operating characteristic curve (SROC; *Figure 4*). This visually elucidates the trade-off between sensitivity and specificity across the three population-based study samples that assessed MSI (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] In this SROC (*Figure 4*), the unclassified variants mentioned in Poynter et al. (2008) and Barnetson et al. (2006) were assumed to be negative reference standard results.[31, 52]

Figure 4: SROC graph for MSI testing where UV are negative



Key: Barnetson et al. (2006), solid black circles[52]; Southey et al. (2005), solid grey circles[54]; Poynter et al. (2008), no fill[31]; +, MSI-L is positive, -, MSI-L is negative; MSI, microsatellite instability; SROC, summary receiver operating characteristic; UV, unclassified variants

The ideal diagnostic test would generate a point in the upper left corner of the ROC space, representing 100% sensitivity (no false negatives) and 100% specificity (no false positives). The closest point to this is Barnetson et al. (2006) with MSI-L as positive.[52] When MSI-L is considered negative, the specificity for Barnetson et al. (2006) improves slightly, but the sensitivity is reduced.[52] This was also the case for Southey et al. (2005), however, due to the wider discrepancy between sensitivity and specificity, the points are further away from perfect classification.[54] With regard to Poynter et al. (2008), the lack of false negatives ensured sensitivity remained constant, with only specificity altering according to allocation of MSI-L results.[31]

It is unsurprising that, on the whole, sensitivity was higher and specificity lower when MSI-L was considered to be a positive result compared to when MSI-L was considered to be a negative result; including MSI-L as a positive result essentially lowers the threshold for a positive index test result. Further to the results presented above, Barnetson et al. (2006) also report that MSI-H has a sensitivity of 83% for the detection of MLH1 mutations, 75% for the detection of *MSH2* mutations, and 17% for the detection of *MSH6* mutations, whereas MSI-L had sensitivities of 17%, 25%, and 50%, respectively.[52] Therefore, the usefulness of including MSI-L as a positive index test result will likely vary according to which gene is

mutated. Indeed, according to Mueller et al. (2009), the vast majority of mutations detected are usually in the *MSH2* or *MLH1* genes, followed by *MSH6* and finally *PMS2*, but it is not clear what thresholds were used in this study to define MSI-H, MSI-L and MSS. Similarly, Caldes et al. (2004) suggest that *MSH6* does not always produce instability in tumours but in this study an MSI-L categorisation is not used (see *Table 10*, *Section 2.2.5.1.1)*.[55]

For the five single-gate, high-risk samples presented in *Table 13* (Caldes, 2004; Mueller, 2009; Overbeek, 2007; Poynter, 2008; Shia, 2005),[31, 55-58] sensitivity estimates ranged from 79.4% (95% CI 62.1, 91.3) in Caldes et al. (2004)[55] to 100.0% (85.8, 100.0) in Shia et al. (2005)[58] when MSI-L was considered to be an index test positive result. Therefore, all of these sensitivity estimates were >79%. It is important to remember that for three of these studies (Caldes, 2004; Overbeek, 2007; Shia, 2005) MSI was already categorised bi-modally, and therefore, the estimates presented in *Table 13* are identical to those presented in *Table 11* (and are based upon the same data).[55, 57, 58] For the other two single-gate, high-risk studies (Mueller, 2009; Poynter, 2008), sensitivities were very similar, albeit slightly higher when MSI-L was considered to be positive (93.1% (95% CI 77.2, 99.2) for Mueller, 2009; 94.7% (95% CI 82.3, 99.4 for Poynter, 2008) compared to when MSI-L was considered to be negative (see *Table 11*, *Section 2.2.5.1.2* above, 91.3% (95% CI 72.0, 98.9 for Mueller, 2009; 86.8% (95% CI 71.9, 95.6) for Poynter, 2008).[31, 56] The MSI data from the reference standard positive study (Hendriks, 2003) was also used to generate sensitivity estimates (*Table 13*) and, similarly, when MSI-L cases were considered to be index test positives, sensitivity was slightly higher (92.0% [95% CI 74.0, 99.0]) than when MSI-L cases were considered to be index test negatives (88.0% [95% CI 68.8, 97.5]).[59]

Other test accuracy estimates

Likelihood ratios (LR+ and LR−), PPV and NPV were calculated for the three population-based studies reporting MSI data (Poynter, 2008; Barnetson, 2006; Southey, 2005) when MSI-L was assumed to be a positive index test result (and the unclassified variants reported in Barnetson et al. (2006) and Poynter et al. (2008) were assumed to be negative reference standard results).[31, 52, 54] These estimates are summarised in *Table 14*.

Table 14: LR+, LR−, PPV and NPV for MSI; MSI-H+MSI-L vs MSS

Author, year	LR+	LR−	PPV (%)	NPV (%)
Single-gate, population-based samples				
Poynter, 2008[a; 31]	1.42 (1.35, 1.50)	0.00 (NE)[b]	12.6 (9.8, 16.0)	100.0 (97.9, 100.0)
Barnetson, 2006[52]	6.01 (4.58, 7.89)	0.08 (0.02, 0.30)	35.9 (25.3, 47.6)	99.3 (97.4, 99.9)
Southey, 2005[54]	2.28 (1.56, 3.33)	0.09 (0.01, 0.65)	50.0 (32.4, 67.6)	96.0 (79.6, 99.9)

Notes: [a] Population based sample; [b] Not estimable

For all three population-based studies, LR+ was reduced when MSI-L was considered to be a positive index test result (1.42 [95% CI 1.35, 1.50] for Poynter, 2008; 6.01 [95% CI 4.58, 7.89] for Barnetson, 2006; 2.28 [95% CI 1.56, 3.33] for Southey, 2005) compared to when MSI was considered to be a negative test result (2.57 [95% CI 2.32, 2.85] for Poynter, 2008; 8.94 [95% CI 5.54, 14.20] for Barnetson, 2006; 5.92 [95% CI 2.48, 14.10] for Southey, 2005).[31, 52, 54] As before, LR− could only be estimated for two of the studies (Barnetson,

2006; Southey, 2005) because there were no false negative MSI results for Poynter et al. (2008).[31, 52, 54] Again, LR− was similar in both studies (Barnetson, 2006; Southey, 2005; *Table 14*) and was lower when MSI-L was considered to be a positive index test result (0.08 [95% CI 0.02, 0.30] for Barnetson, 2006; 0.09 [95% CI 0.01, 0.65] for Southey, 2005) compared with when MSI-L was considered to be a negative index test result (0.36 [95% CI 0.21, 0.60] for Barnetson, 2006; 0.32 [95% CI 0.15, 0.67] for Southey, 2005).[52, 54]

As before, PPV estimates (the probability of someone with a positive result actually having Lynch syndrome as defined by the reference standard) varied a lot more between these studies with the lowest estimate still coming from Poynter et al. (2008) at 12.6% (95% CI 9.8, 16.0) and the highest still coming from Southey et al. (2005) at 50.0% (95% CI 32.4, 67.6).[31, 54] For all three studies, PPV estimates were lower when MSI-L was considered to be an index test positive (*Table 14*) than when MSI-L was considered to be an index test negative (*Table 12*). Conversely, NPV (the probability of someone with a negative test result actually not having Lynch syndrome as defined by the reference standard), which was consistent across these three studies (with all estimates >96%), was higher when MSI-L was considered to be an index test positive (*Table 14*) than when MSI-L was considered to be an index test negative (*Table 12*). However, it should be noted that for the unselected CRC population in Poynter et al. (2008), the NPV estimate was 100% regardless of whether MSI-L was considered to be a positive or negative index test result, although confidence intervals were slightly wider when MSI-L was considered to positive (95% CI 97.9, 100.0) compared to negative (95% CI 99.0, 100.0).[31]

Secondary analyses (unclassified variants as index test positives)

As before, secondary analyses were conducted where unclassified variants were considered to indicate a positive reference standard test result. Although five of the included studies (the population-based sample in Poynter, 2008; Barnetson, 2006; Caldes, 2004; Shia, 2005; Hendriks, 2003)[31, 52, 55, 58, 59] reported unclassified variants in their assessment of MSI, only one (Hendriks, 2003)[59] provided sufficient data to conduct secondary analyses. However, because this study is a reference standard positive study, only sensitivity estimates were made.

Thus, when unclassified variants were considered to be reference standard positives, and MSI-L was considered to be an index test positive, Hendriks et al. (2003) reported sensitivity as 93.9% (95% CI 80.3, 98.3).[59] As expected, because the threshold for an MSI case is essentially lowered when MSI-L is considered to be positive, this sensitivity was higher than that reported when MSI-L was considered to be an index test negative and unclassified variants were considered to be reference standard positives (84.8% [95% CI 69.0, 93.3]). When comparing the results from Hendriks et al. (2003) where unclassified variants were considered to be reference standard positives (MSI-L as an index test positive) with those generated when the unclassified variants were considered to be reference standard negatives (MSI-L as an index test positive), sensitivity was very similar: 93.9% (95% CI 80.3, 98.3) when unclassified variants were positive, 92.0% (95% CI 74.0 to 99.0) when unclassified variants were negative.[59]

2.2.5.2 Assessment of test accuracy (IHC)

IHC was conducted in all of the 10 studies (11 samples) included in the review of test accuracy. However, not all studies provided sufficient data to be included in analyses.

Indeed, in two study samples (the high-risk sample in Poynter, 2008; Mueller 2009),[31, 56] despite IHC being conducted, insufficient data were provided for these samples to be included in any of the IHC analyses. In five cases (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012)[31, 52, 54, 59, 60] the analyses for IHC were split according to the particular protein assessed (MLH1, MSH2, MSH6 or PMS2), enabling an assessment of IHC for at least one of these individual proteins (i.e., whether an absence of a particular protein accurately identifies a mutation in a particular gene). In seven cases (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks)[52-55, 57-59] an overall result is given (i.e., whether a positive IHC result, regardless of which protein this applies to, predicts a positive reference standard result).

As with the results for MSI, in primary analyses unclassified variants are considered to be reference standard negatives. Where sufficient data were available, secondary analyses were conducted where unclassified variants were considered to be reference standard positives.

2.2.5.2.1 Overall IHC results

As noted above, seven studies (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks, 2003) provided sufficient data to enable an assessment of the overall test performance of IHC (i.e., whether a positive IHC result, regardless of which protein this applies to, predicts a positive reference standard result).[52-55, 57-59] All of these studies assessed MLH1, MSH2 and MSH6 proteins. Therefore, abnormal staining for any of these three proteins was considered to be a positive index test result. However, Southey et al. (2005) and Overbeek et al. (2007) also assess PMS2. So for these two studies an abnormal PMS2 result would also be included as a positive index test result.[54, 57]

Sensitivity and specificity estimates

Three population-based studies, all based on age-limited samples (Barnetson, 2006; Limburg, 2011; Southey, 2005) were included in primary analyses, where unclassified variants were considered to be reference standard negatives.[52-54] For all three of these studies sensitivity estimates were made, with Limburg et al. (2011) providing the lowest estimate (85.7%; 95% CI 41.2, 99.6) and Southey et al. (2005) providing the highest estimate 100.0% (81.5% to 100.0%).[53, 54] The study by Southey et al. (2005) included an assessment of PMS2 as well as MLH1, MSH2 and MSH6, and it is possible that this accounted for the higher sensitivity estimate.[54] Nevertheless, all sensitivity estimates from the population-based studies were >85% (*Table 15*). For two of the population-based studies (Limburg, 2011; Southey, 2005) specificity estimates were also made for overall IHC results and were >80% (91.9% [95% CI 86.3, 95.7] for Limburg, 2011 and 80.5% [95% CI 65.1, 91.2] for Southey, 2005; *Table 15*).[53, 54] Specificity could not be estimated for the third population-based study (Barnetson, 2006) because overall IHC results were only available for reference standard positive participants.[52]

Table 15: Sensitivity and specificity for IHC (overall results)

Author, year	Sensitivity (%)	Specificity (%)
Single-gate, population-based samples		
Barnetson, 2006[52]	92.6 (76.6, 97.9)	NE[a]
Limburg, 2011[53]	85.7 (42.1, 99.6)	91.9 (86.3, 95.7)
Southey, 2005[54]	100.0 (81.5, 100.0)	80.5 (65.1, 91.2)
Single-gate, high-risk samples		
Caldes, 2004[55]	96.4 (81.7, 99.9)	—
Overbeek, 2007[57]	87.5 (52.9, 97.7)	—
Shia, 2005[58]	80.8 (60.6, 93.4)	—
Reference standard positive study sample		
Hendriks, 2003[59]	91.7 (77.5, 98.2)	—

Notes: [a] Not estimable

For the three high-risk, single-gate samples presented in *Table 15* (Caldes, 2004; Overbeek, 2007; Shia, 2005), sensitivity estimates ranged from 80.8% (95% CI 60.6, 93.4) in Shia (2005) to 96.4% (95% CI 81.7, 99.9) in Caldes (2004).[55, 57, 58] Two of these high-risk, single gate studies mention unclassified variants (Caldes, 2004; Shia, 2005) and these are counted as reference standard negatives in these analyses.[55, 58] The data from the reference standard positive study that assessed overall IHC results (Hendriks, 2003) was also used to generate a sensitivity estimate (91.7%; 95% CI 77.5, 98.2).[59]

Due to a potential for spectrum bias, it would be expected that studies recruiting high-risk populations would result in higher sensitivity estimates than those estimated from population-based studies. However, as discussed in a systematic review by Palomaki et al. (2009), and as with the MSI results reported above (*Section 2.2.5.1*), we did not find great differences between the sensitivity estimates in the population-based studies and the high-risk studies.[39] However, this could be because the three population-based studies with overall IHC data (Barnetson, 2006; Limburg, 2011; Southey, 2005) are based on age-limited populations and may also be subject to spectrum bias.[52-54]

Other test accuracy estimates

The other test accuracy outcomes included in this review were likelihood ratios (LR+ and LR−), PPV and NPV, accuracy or concordance with the reference standard, diagnostic yield, and test failure rates. As previously mentioned, the latter three outcomes (accuracy or concordance with the reference standard, diagnostic yield and test failure rates were not reported in any of the included studies). The other four outcomes (LR+, LR−, PPV and NPV) were calculated for the two population-based studies with sufficient available overall IHC data (Limburg, 2011; Southey, 2005).[53, 54] These outcomes are reported in *Table 16*. Unclassified variants are considered to be reference standard negative results. Again, Barnetson et al. (2006) is not included here because overall IHC results were only available for reference standard positive participants.[52]

Table 16: Likelihood ratios and predictive values for IHC (overall results)

Author, year	LR+	LR−	PPV (%)	NPV (%)
Single-gate, population-based samples				
Limburg, 2011[53]	10.6 (5.7, 19.7)	0.16 (0.02, 0.95)	33.3 (13.3, 59.0)	99.3 (96.0, 100.0)
Southey, 2005[54]	5.1 (2.8, 9.5)	0.00 (NE)[a]	69.2 (48.2, 85.7)	100.0 (89.4, 100.0)

Notes: [a] Not estimable

LR+ was 10.6 (95% CI 5.7, 19.7) for Limburg et al. (2011) and 5.1 (95% CI 2.8, 9.5) for Southey et al. (2005).[53, 54] LR− could only be estimated for Limburg (2011) because there were no false negative overall IHC results in Southey et al. (2005).[53, 54] LR− was estimated to be 0.16 (95% CI 0.02, 0.95) in Limburg (2011).[53] PPV (the probability of someone with a positive result actually having Lynch syndrome as defined by the reference standard) was lower in Limburg et al. (2011) at 33.3% (95% CI 13.3, 59.0) than in Southey et al. (2005) at 69.2% (95% CI 48.2, 85.7).[53, 54] NPV (the probability of someone with a negative test result actually not having Lynch syndrome as defined by the reference standard) was high in both studies: both estimates were >99% (*Table 16*). Again, where apparent differences in IHC performance exist between these two studies (for example in PPV results) it should be considered that Southey et al. (2005) included an assessment of PMS2 in their results whereas Limburg et al. (2011) did not.[53, 54] Additionally, the specific techniques and methods used to perform the reference standard differ between studies (see *Table 8*) and this may also impact upon apparent test performance.

Secondary analyses (unclassified variants as index test positives)

Secondary analyses were conducted where unclassified variants were considered to indicate a positive reference standard test result. It was not possible to include all studies reporting IHC data in these analyses (because sufficient data were not reported). Indeed, only two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary analyses.[55, 59] However, because Caldes et al. (2004) was based on a high-risk population and Hendriks (2003) is a reference standard positive study, only sensitivity estimates were made.[55, 59]

Thus, when unclassified variants were considered to be reference standard positives, data from Caldes et al. (2004) estimated overall IHC sensitivity as 75.0% (95% CI 57.8, 87.9) and data from Hendriks et al. (2003) estimated overall IHC sensitivity as 88.6% (95% CI 76.0, 95.0).[55, 59] For Caldes et al. (2004) this represents quite a reduction in sensitivity compared to when unclassified variants were considered to be index test negatives (96.4%; 95% CI 81.7, 99.9), whereas for Hendriks et al. (2003), sensitivity was only slightly reduced by categorising unclassified variants as reference standard positives compared rather than negatives (*Table 15*).[55, 59]

2.2.5.2.2 IHC according to protein

The analyses above (where overall IHC results are considered) are limited in what they can demonstrate about loss of expression for individual proteins and how this relates to pathogenic mutations. Indeed, Overbeek et al. (2007) note that tumour cells of *MLH1* mutation carriers generally lacked MLH1 and PMS2 protein by IHC staining, those of *MSH2* mutation carriers lacked MSH2 and MSH6, those of *MSH6* mutation carriers lacked MSH6,

and those of *PMS2* mutation carriers lacked PMS2.[57] Furthermore, Barnetson et al. (2006) suggest that "the absence of MSH6 protein predicted mutations in *MSH2* or *MSH6 […]*, as did the absence of MSH2 for mutations in *MSH2* or *MSH6 […]*, reflecting the biologic interaction between these proteins."[52]

Indeed, the significance of the patterns of IHC abnormality in predicting underlying genetic causes of CRC predisposition appears to be becoming clearer, if more complex (*Table 4, Section 1.2.1.1.2*); it is becoming apparent that not all mutations are associated with loss or abnormality of the corresponding protein, and that specific IHC abnormality cannot be taken for an absolute indicator of the underlying genetic defect.[34] However, it was beyond the scope of this review to use data from the included studies to attempt an assessment of which IHC protein results were more or less likely to predict which pathogenic mutations (or whether particular patterns or combinations of IHC abnormality correspond to particular defects). In any case, there was insufficient individual patient data available from the population-based samples that could be used to attempt such an analysis.

However, for five study samples (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) sufficient data were available to enable an assessment of IHC for at least one individual protein, in terms of whether loss of expression in that protein was an accurate test of a pathogenic mutation in that gene (regardless of whether there was also loss of expression in additional proteins).[31, 52, 54, 59, 60] Three of these studies provided sufficient data to assess whether a loss of protein expression in MLH1, MSH2 and MSH6 (Barnetson, 2006; Southey, 2005; Hendriks, 2003) was an accurate test of a pathogenic mutation in the same gene. Southey et al. (2005) also provided these data, and additionally provided data to enable an assessment of whether loss of protein expression in PMS2 was an accurate test of a pathogenic mutation in *PMS2*.[52, 54, 59] The study by Okkels et al. (2006) was designed to assess whether lack of protein expression in MSH6 would predict a pathogenic mutation in *MSH6*.[60] For the population-based sample in Poynter et al. (2008), limited IHC data were available for individual proteins.[31] However, sufficient data were available to assess the specificity of a loss of protein expression in MLH1. Sensitivity was not calculated for this study because these data were only available for reference standard negatives. The estimates of sensitivity and specificity generated from these studies (for IHC of individual proteins) are provided in *Table 17*.

For the four studies that provided data relevant to whether loss of expression in MLH1 was an accurate test result for assessing a pathogenic mutation in *MLH1*, three were population-based, single-gate studies (Barnetson, 2006; Southey, 2005; Poynter, 2008)[31, 52, 54] and one was a reference standard positive study (Hendriks, 2003).[59] The studies by Barnetson et al. (2006), Southey et al. (2005) and Hendriks et al. (2003) all provided data from which sensitivities were generated.[52, 54, 59] These ranged from 50.0% (95% CI 26.0, 74.0) for Southey et al. (2005) to 100.0% (95% CI 73.5, 100.0) for Barnetson et al. (2006).[52, 54] The three population-based studies (Barnetson, 2006; Southey, 2005; Poynter, 2008) provided data from which specificities were generated.[31, 52, 54] These ranged from 70.6% (95% CI 66.8, 74.2) for Poynter et al. (2008) to 96.0% (95% CI 93.1, 97.9) for Barnetson et al. (2006).[31, 52] The results for MSH2 were even more variable. Three studies provided data relevant to whether loss of expression in MSH2 was an accurate test result for assessing a pathogenic mutation in *MSH2*. Two of these were single-gate, population-based studies (Barnetson, 2006; Southey, 2005)[52, 54] and one was a reference standard positive study (Hendriks,

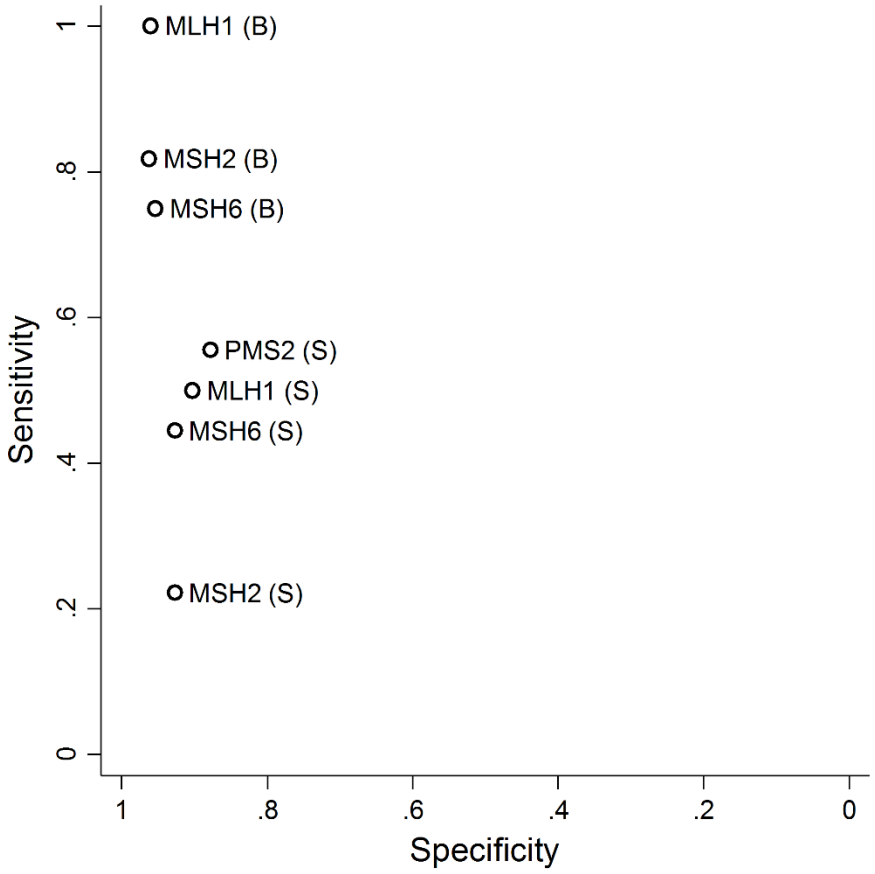
2003).[59] Sensitivities ranged from 22.2% (95% CI 6.4, 47.6) for Southey (2005) to 81.8% (95% CI 48.2, 97.7) for Barnetson et al. (2006) and Hendriks et al. (2003).[52, 54, 59] The two population-based studies (Barnetson, 2006; Southey, 2005) provided data from which specificities were generated.[52, 54] These were fairly consistent across both studies, with both specificity estimates being >92% (*Table 17*).

Four studies provided data relevant to whether loss of expression in MSH6 was an accurate test result for assessing a pathogenic mutation in *MSH6*. Two of these were single-gate, population-based studies (Barnetson, 2006; Southey, 2005),[52, 54] and two were reference standard positive studies (Hendriks, 2003; Okkels, 2012).[59, 60] Again, there was more variation in the sensitivities generated than in the specificities: sensitivities ranged from 44.4% (95% CI 21.5, 69.2) for Southey (2005) to 75.0% (95% CI 19.4, 99.4) for Barnetson et al. (2006) and Hendriks et al. (2003), whereas specificities, which were only generated for the population-based studies (Barnetson, 2006; Southey, 2005) were similar across studies, with both studies producing an estimate >92% (*Table 17*).[52, 54, 59] Only one study (Southey, 2005) provided data to enable an assessment of whether loss of expression in PMS2 was an accurate test result for assessing a pathogenic mutation in *PMS2* (*Table 17*), providing a sensitivity estimate of 55.6% (95% CI 30.8, 78.5) and a specificity estimate of 87.8 (95% CI 73.8, 95.9).[54]

With the exception of the sensitivity estimates generated from the study by Southey et al. (2005), it appears that the data presented in *Table 17* is fairly consistent across studies.[54] This difference in Southey et al. (2005) appears to be due to higher rates of false negative results than in the other studies.[54] It is possible that this is due to specific between-study differences in the assessment of IHC (a positive IHC result is a somewhat subjective judgement, made by human assessors, so interrater variability may impact upon results). Again, it is also possible that between-study differences in the reference standard could, to some extent, account for these differences in sensitivity estimates.

This is further demonstrated in *Figure 5* where sensitivities and specificities from Barnetson et al. (2006) and Southey et al. (2005) are graphically summarised using a receiver operating characteristic curve (SROC).[52, 54] This visually elucidates the trade-off between sensitivity and specificity across these two population-based study samples for each of the individual proteins assessed (MLH1, MSH2 and MSH6 as well as PMS2 for Southey, 2005).[54] In this figure, unclassified variants were assumed to be negative reference standard results.

Figure 5: SROC graph for IHC testing where UV are negative



Key: Barnetson et al. (2006), (B)[52]; Southey et al. (2005), (S)[54]

Table 17: Sensitivity and specificity of IHC according to lack of protein expression

Author, year	MLH1		MSH2		MSH6		PMS2	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Single-gate, population-based samples								
Poynter, 2008[31]	—	70.6 (66.8, 74.2)	—	—	—	—	—	—
Barnetson, 2006[52]	100.0 (73.5, 100.0)	96.0 (93.1, 97.9)	81.8 (48.2, 97.7)	96.2 (93.5, 98.0)	75.0 (19.4, 99.4)	95.4 (92.5, 97.4)	—	—
Southey, 2005[54]	50.0 (26.0, 74.0)	90.2 (76.9, 97.3)	22.2 (6.4, 47.6)	92.7 (80.1, 98.5)	44.4 (21.5, 69.2)	92.7 (80.1, 98.5)	55.6 (30.8, 78.5)	87.8 (73.8, 95.9)
Reference standard positive study samples								
Hendriks, 2003[59]	85.7 (63.7, 97.0)	—	81.8 (48.2, 97.7)	—	75.0 (19.4, 99.4)	—	—	—
Okkels, 2012[60]	—	—	—	—	72.7 (51.8, 86.8)	—	—	—

Other test accuracy estimates

The other test accuracy outcomes included in this review were likelihood ratios (LR+ and LR−), PPV and NPV, accuracy or concordance with the reference standard, diagnostic yield, and test failure rates. As previously mentioned, the latter three outcomes (accuracy or concordance with the reference standard, diagnostic yield and test failure rates were not reported in any of the included studies). The other four outcomes (LR+, LR−, PPV and NPV) were calculated for the two population-based studies with sufficient available IHC data by protein (Barnetson, 2006; Southey, 2005).[52, 54]

LR+, and LR− are reported in *Table 18*. Unclassified variants were considered to be reference standard negative results. For loss of protein expression in MLH1, LR+ was 25.0 (95% CI 14.4, 43.5) for Barnetson et al. (2006) and 5.1 (95% CI 1.8, 14.5) for Southey et al. (2005).[52, 54] For loss of protein expression in MSH2, LR+ was 21.6 (95% CI 11.6, 40.2) for Barnetson et al. (2006) and 3.0 (95% CI 0.8, 12.2) for Southey et al. (2005).[52, 54] For loss of protein expression in MSH6, LR+ was 16.2 (95% CI 7.6, 34.3) for Barnetson et al. (2006) and 6.1 (95% CI 1.8, 20.3) for Southey et al. (2005).[52, 54] For loss of protein expression in PMS2, data were only available from Southey et al. (2005), and LR+ was estimated as 4.6 (95% CI 1.8, 11.4).[54] There are several possible reasons why LR+ estimates were higher in Barnetson et al. (2006) than in Southey et al. (2005) including the fact that Barnetson et al. (2006) was a larger study than Southey et al. (2005), that the reference standard was not identical in these studies, and that there is a possibility that IHC ratings may have differed across studies (interrater reliability).[52, 54] For loss of protein expression in MLH1, LR− was only estimated for one study (Southey, 2005; 0.6, 95% CI 0.4, 0.9) because there were no false negative results in Barnetson et al. (2006).[52, 54]

For loss of protein expression in MSH2, LR− was 0.2 (95% CI 0.1, 0.7) for Barnetson et al. (2006) and 0.8 (95% CI 0.7, 1.1) for Southey et al. (2005).[52, 54] For loss of protein expression in MSH6, LR− was 0.3 (95% CI 0.1, 1.4) for Barnetson et al. (2006) and 0.6 (95% CI 0.4, 0.9) for Southey et al. (2005). For loss of protein expression in PMS2, data were only available from Southey et al. (2005), and LR− was estimated as 0.5 (95% CI 0.3, 0.9).[54]

Table 18: Likelihood ratios for IHC, according to loss of protein expression

Author, year	MLH1		MSH2		MSH6		PMS2	
	LR+	LR−	LR+	LR−	LR+	LR−	LR+	LR−
Barnetson, 2006[52]	25.0 (14.4, 43.5)	0.0 (NE) [a]	21.6 (11.6, 40.2)	0.2 (0.1, 0.7)	16.2 (7.6, 34.3)	0.3 (0.1, 1.4)	—	—
Southey, 2005[54]	5.1 (1.8, 14.5)	0.6 (0.4, 0.9)	3.0 (0.8, 12.2)	0.8 (0.7, 1.1)	6.1 (1.8, 20.3)	0.6 (0.4, 0.9)	4.6 (1.8, 11.4)	0.5 (0.3, 0.9)

Notes: [a] Not estimable

Table 19: PPV and NPV according to loss of protein expression

Author, year	MLH1		MSH2		MSH6		PMS2	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
Barnetson, 2006[52]	50.0 (29.1, 70.9)	100.0 (98.7, 100.0)	42.9 (21.8, 66.0)	99.3 (97.7, 99.9)	16.7 (3.6, 41.4)	99.7 (96.2, 100.0)	—	—
Southey, 2005[54]	69.2 (38.6, 90.9)	80.4 (66.1, 90.6)	57.1 (18.4, 90.1)	73.1 (59.0, 84.4)	72.7 (39.0, 94.0)	79.2 (65.0, 89.5)	66.7 (38.4, 88.2)	81.8 (67.3, 91.8)

PPV (the probability of someone with a positive result actually having Lynch syndrome as defined by the reference standard) and NPV (the probability of someone with a negative test result actually not having Lynch syndrome as defined by the reference standard) are reported, according to loss of protein expression, in *Table 19*. Again, unclassified variants were considered to be reference standard negative results. For loss of protein expression in MLH1 and MSH2, the PPV and NPV results were largely consistent across the two studies providing data (Barnetson, 2006; Southey, 2005; *Table 19*) although PPV estimates for these two genes were lower in Barnetson et al. (2006) and NPV estimates for these two genes were lower in Southey et al. (2005).[52, 54] For loss of expression in MSH6, NPV estimates were consistent across the two studies (*Table 19*), but PPV estimates were vastly different, with the data from Barnetson et al. (2006) resulting in a PPV of 16.7 (95% CI 3.6, 41.4) and the data from Southey et al. (2005) resulting in a PPV of 72.7 (95% CI 39.0, 94.0).[52, 54] Although the reason for this difference is not completely clear, it is likely due, at least in part, to the very low number of true positive results (n=3) for loss of expression in MSH6 in the study by Barnetson et al. (2006).[52] Again, only Southey et al. (2005) provided data for loss of protein expression in PMS2, and PPV was estimated as 66.7 (95% CI 38.4, 88.2) and NPV as 81.8 (95% CI 67.3, 91.8).[54]

Secondary analyses (unclassified variants as index test positives)

Secondary analyses were conducted where unclassified variants were considered to indicate a positive reference standard test result. It was not possible to include all five studies (Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) reporting IHC data for individual proteins in these analyses because sufficient data were not always reported.[31, 52, 54, 59, 60] Indeed, only one study (Hendriks, 2003) provided sufficient data to conduct secondary analyses and, because this study is a reference standard positive study, only sensitivity estimates were made.[59] These sensitivity estimates were very similar to those estimated from data where unclassified variants were considered to be reference standard negatives.

Indeed, when unclassified variants were considered to be reference standard positives, loss of protein expression in MLH1 was estimated to have a sensitivity of 80.0% (95% CI 60.8, 91.1) compared with the previously reported sensitivity of 81.0% (95% CI 58.1, 94.6) when unclassified variants were considered to be index test negatives. Loss of protein expression in MSH2 was estimated to have a sensitivity of 83.3% (95% CI 55.1, 95.3) compared with the previously reported sensitivity of 81.8% (95% CI 48.2, 97.7) when unclassified variants were considered to be index test negatives. Loss of protein expression in MSH6 was estimated to have a sensitivity of 80.0% (95% CI 37.6, 96.4) compared with the previously reported sensitivity of 75.0% (95% CI 19.4, 99.4) when unclassified variants were considered to be index test negatives (*Table 17*).

2.2.6 Summary of results from the test accuracy review

2.2.6.1 Summary of included studies

Ten studies met the test accuracy review inclusion criteria. One of the included studies had two distinct samples (a population-based sample and a high-risk sample) and these two samples are treated separately. Thus, although there are 10 included studies, there are 11 included populations/data sets. The results from all 11 populations are considered.

The 11 study samples have been divided as follows: four single-gate studies with population-based samples, including one apparently unselected CRC population (Poynter, 2008)[31] and three age-limited populations (Barnetson 2006, Limburg, 2011, Southey 2005);[52-54] five single-gate studies based on high-risk populations (Caldes 2004, Mueller 2009, Overbeek 2007, Poynter 2008, Shia 2005);[31, 55-58] and two studies that were a variation on a two-gate study design (Hendriks 2003, Okkels 2012)[59, 60] where participants with positive reference standard results were recruited but no reference standard negatives were recruited. For this report, and for clarity, these studies have been termed reference standard positive studies.

With the exception of the studies by Limburg et al. (2011) and Okkels et al. (2012), all studies assessed MSI.[53, 60] Although IHC was conducted in all of the 10 studies (11 samples) included in the review of test accuracy, not all studies provided sufficient data to be included in analyses. Indeed, in two study samples (the high-risk sample in Poynter, 2008 and Mueller 2009), despite IHC being conducted, insufficient data were provided for these samples to be included in any of the IHC analyses.[31, 56] None of the studies made a direct comparison of MSI and IHC. As such, results are reported separately for these tests.

There was significant methodological and clinical heterogeneity across studies. In particular, the reference standard differed between studies, as did the index tests. With regard to the reference standard there were differences in the testing methods used (including sequencing methods and genes tested, techniques used to test for large genomic alterations and deletions, genes tested for large genomic alterations and deletions, and whether unclassified variants were investigated). As a result of this, pooling of data in statistical analyses was not appropriate. In addition, there were insufficient data to conduct subgroup analyses based on most of these variables. However, test performance statistics were primarily generated with unclassified variants categorised as negative reference standard results, and two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary analyses where unclassified variants were categorised as positive reference standard results. [55, 59]

Quality appraisal was conducted, using Phase 3 of the QUADAS-2 tool, for all 11 data sets (all 10 studies, including both the population-based and high-risk samples reported in Poynter, 2008).[31] Four of the studies were rated as having a low risk of bias due to patient selection, three of these were population-based single-gate studies (Barnetson, 2006; Limburg, 2011; Southey, 2005)[52-54] and the other was a reference standard positive study (Okkels, 2012)[60] for which only sensitivity estimates could be made. For all studies included in the review, there were no concerns about whether or not the included participants matched the review question. For both index tests, all studies were rated as unclear with regards to whether the conduct and interpretation of the test could have introduced bias but there were no concerns (in any of the studies) that the conduct or interpretation of either of the index tests was different from the review question. All of the included studies, apart from Hendriks et al. (2003), were rated as unclear with regards to whether or not the conduct or

interpretation of the reference standard could have introduced bias.[59] This was because only Hendriks et al. (2003) specified that the reference standard results were interpreted without knowledge of the results of the index test, with the rest of the studies not reporting this information.[59] However, in all of the included studies the reference standard was assessed as likely to correctly classify the target condition (because a genetic definition of Lynch syndrome is being used in this review) and there were no concerns, in any of the studies, that the target condition, as defined by the reference standard, did not match the review question. For all included studies, it was unclear whether the flow of participants through the study could have introduced bias.

The index tests included in this review (MSI and IHC) are highly susceptible to spectrum effects in populations that have been selected due to clinical characteristics. In particular, increased presence of MMR mutation carriers in a population would change the apparent sensitivity and specificity of the index tests (Barnetson, 2006).[52] However, a previous review, did not find that this issue led to significant bias in estimates of sensitivity (Palomaki, 2009).[39] Due to this, studies recruiting high-risk populations have only been used to estimate sensitivity. For the four samples included in this review that can be described as population-based samples (Poynter, 2008; Barnetson, 2006; Limburg, 2011; Southey, 2005) sensitivity, specificity, LR+, LR−, PPV and NPV have all been estimated.[31, 52-54] However, it should be noted that the latter three studies recruited age-limited populations (Barnetson, 2006; Limburg, 2011; Southey, 2005) for which some spectrum bias may be expected.[52-54]

2.2.6.2 Summary of results for MSI

A variety of between-study differences exist in the MSI testing procedures used. In addition, differences between studies in MSI testing methods were not always clear because methods were not always reported in sufficient detail. For example, three of the eight studies assessing MSI (Poynter, 2008; Mueller, 2009; Overbeek, 2007) did not report microdissection techniques (microdissection assists in assuring that malignant tissue that does not contain DNA from surrounding, healthy colonic tissue is analysed).[31, 56, 57] The other differences between studies in MSI testing methods can be categorised as: differences in the panel of markers used, differences in the way in which MSI was categorised (e.g., as a bimodal or trimodal distribution), and differences in the thresholds used to categorise MSI. Indeed, none of the population-based studies assessed the same panel of markers (differences exist in both the type and number of markers). Five studies (Barnetson, 2006; Southey, 2005; Poynter, 2008; Mueller, 2009; Hendriks, 2003)[31, 52, 54, 56, 59] define tumours as MSI-H, MSI-L or MSS, also known as a trimodal distribution, two (Overbeek, 2007; Shia, 2005)[57, 58] define tumours as MSI positive or negative, also known as a bimodal distribution, and one (Caldes, 2004)[55] uses a bimodal distribution but defines tumours as either MSI-H or MSS and studies Of the five studies that use the MSI-H, MSI-L and MSS categories (trimodal distribution), the thresholds used to categorise the tumours vary greatly, with one of these studies using the commonly used threshold of more than 30% of unstable markers to define MSI-H tumours (Poynter, 2008), three studies using differing numbers of unstable markers to define MSI-H tumours, and one study (Mueller, 2009) not providing details on the thresholds used to categorise the tumours as MSI-H, MSI-L and MSS.[31, 56] Of the two studies that defined tumours as positive or negative (bimodal distribution), one reported using a threshold of more than 30% of unstable markers to define MSI positive tumours (Shia, 2005) and the other defined MSI positive tumours as those with more than two unstable Bethesda panel markers (Overbeek, 2007).[57, 58] The study by Caldes (2004) defined MSI-H tumours as

those with two or more unstable Bethesda markers (or one marker in the case of BAT26), and MSS tumours as those showing no instability.[55] It is not clear how cases with only one unstable marker (other than BAT26) were categorised, but in any case data are only presented for tumours that were categorised as MSS and MSI-H.

In primary analyses unclassified variants were categorised as negative reference standard results. Six study samples provided data where MSI-L was considered to be a negative index test result (both samples in Poynter, 2008; Barnetson, 2006; Southey, 2005; Mueller, 2009; Hendriks, 2003).[31, 52, 54, 56, 59] The other three samples utilised a bimodal distribution of MSI (Overbeek, 2007; Shia, 2005; Caldes, 2004).[55, 57, 58] Across all nine samples, when MSI-L was considered to be a negative index test result, sensitivity ranged from 66.7% (95% CI 47.2, 82.7) for the population-based sample reported by Barnetson et al. (2006) to 100.0% (95% CI 93.9, 100.0 for the population-based sample in Poynter, 2008 and 95% CI 85.8, 100.0 for the high-risk sample in Shia, 2005).[31, 52, 58] Sensitivity increased when MSI-L was considered to be a positive index test result (for the six study samples where a tri-modal distribution of MSI was used, with data remaining unchanged for the three samples were a bi-modal distribution of MSI was used). Indeed, across the nine study samples, the lower end of the range for sensitivity increased to 79.4% (95% CI 62.1, 91.3) in the high-risk sample recruited by Caldes et al. (2004)[55] with the upper end of the range still being 100%.

In primary analyses (where unclassified variants were categorised as negative reference standard results) three population-based study samples provided data where MSI-L was considered to be a negative index test result (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] Across these three samples, when MSI-L was considered to be a negative index test result, specificity ranged from 61.1% (95% CI 57.0, 65.1) in Poynter (2008) to 92.5% (95% CI 89.1, 95.2) in Barnetson et al. (2006). It should be noted that Barnetson et al. (2006) was based on an age-limited sample whereas Poynter et al. (2008) was based on an unselected CRC population.[31, 52] Specificity decreased when MSI-L was considered to be a positive index test result. Indeed, the lower end of the range decreased to 29.5% (95% CI 25.8, 33.4) in Poynter et al. (2008) and the upper end of the range to 84.5% (95% CI 80.0, 88.2) in Barnetson et al. (2006).[31, 52]

It is unsurprising that, on the whole, sensitivity was higher and specificity lower when MSI-L was considered to be a positive result compared to when MSI-L was considered to be a negative result; including MSI-L as a positive result essentially lowers the threshold for a positive index test result.

For the three studies that recruited population-based samples, LR+, LR−, PPV and NPV were also calculated. LR+ was reduced when MSI-L was considered to be a positive index test result (LR+ 1.42 [95% CI 1.35, 1.50] for Poynter, 2008; LR+ 6.01 [95% CI 4.58, 7.89] for Barnetson, 2006; LR+ 2.28 [95% CI 1.56, 3.33] for Southey, 2005) compared to when MSI was considered to be a negative test result (LR+ 2.57 [95% CI 2.32, 2.85] for Poynter, 2008; LR+ 8.94 [95% CI 5.54, 14.20] for Barnetson, 2006; LR+ 5.92 (95% CI 2.48, 14.10) for Southey, 2005.[31, 52, 54] LR− could only be estimated for two of the studies (Barnetson, 2006; Southey, 2005) because there were no false negative MSI results for Poynter (2008).[31, 52, 54] LR− was similar in both studies and was lower when MSI-L was considered to be a positive index test result (LR− 0.08 [95% CI 0.02, 0.30] for Barnetson, 2006; LR− 0.09 [95% CI 0.01, 0.65] for Southey, 2005) compared with when MSI-L was considered to be a negative index test result (LR− 0.36 [95% CI 0.21, 0.60] for Barnetson, 2006; LR− 0.32 [95% CI 0.15, 0.67] for Southey, 2005).[52, 54] PPV estimates varied a lot more between the studies but were lower

when MSI-L was considered to be an index test positive than when MSI-L was considered to be an index test negative. Conversely, NPV was consistent across these three studies was higher when MSI-L was considered to be an index test positive than when MSI-L was considered to be an index test negative. However, it should be noted that for the unselected CRC population in Poynter et al. (2008), the NPV estimate was 100% regardless of whether MSI-L was considered to be a positive or negative index test result, although confidence intervals were slightly wider when MSI-L was considered to positive (95% CI 97.9, 100.0) compared to negative (95% CI 99.0, 100.0).[31]

Secondary analyses were conducted, where data permitted, where unclassified variants were considered to be positive reference standard results. When MSI-L was considered to be a negative index test result, two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary analysis of sensitivity estimates. Caldes et al. (2004) reported sensitivity as 81.6% (95% CI 65.7, 92.3) and Hendriks et al. (2003) reported sensitivity as 84.8% (95% CI 69.0, 93.3).[55, 59] These results were similar to those obtained when unclassified variants were considered to be negative (79.4% [95% CI 62.1, 91.3] for Caldes, 2004 and 88.8% [95% CI 68.8 to 97.5] for Hendriks, 2003).[55, 59] When MSI-L was considered to be a positive index test result, only one study (Hendriks, 2003) provided sufficient data to conduct a secondary analysis of the sensitivity estimate.[59] In this study, sensitivity was 93.9% (95% CI 80.3, 98.3) which was similar to when unclassified variants were considered to be negative (92.0%; 95% CI 74.0, 99.0).

2.2.6.3 Summary of results for IHC

In primary analyses unclassified variants were categorised as negative reference standard results. Seven study samples (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks 2003) provided data to assess the accuracy of an overall IHC result at identifying a positive reference standard result (i.e., whether a positive IHC result, regardless of which protein this applies to, identifies a positive reference standard result).[52-55, 57-59] Five study samples (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) split IHC data according to the particular protein assessed (MLH1, MSH2, MSH6 or PMS2), enabling an assessment of IHC for at least one of these individual proteins (i.e., whether an absence of a particular protein accurately identifies a mutation in that particular gene).[31, 52, 54, 59, 60]

All of the seven studies (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks 2003) assessing the overall test performance of IHC, assessed MLH1, MSH2 and MSH6 proteins.[52-55, 57-59] Therefore, abnormal staining for any of these three proteins was considered to be a positive index test result. However, Southey et al. (2005) and Overbeek et al. (2007) also assessed PMS2.[54, 57] So for these two studies an abnormal PMS2 result would also be included as a positive index test result. Three of these studies were population-based studies (Barnetson, 2006; Limburg, 2011; Southey, 2005),[52-54] three were single-gate high-risk studies (Caldes, 2004; Overbeek, 2007; Shia, 2005),[55, 57, 58] and one was a reference standard positive study (where only reference standard positives were recruited). Sensitivity estimates ranged from 80.8% (95% CI 60.6, 93.4) in Shia et al. (2005) to 100.0% (95% CI 81.5%, 100.0%) in Southey et al. (2005).[54, 58] The study by Southey et al. (2005) included an assessment of PMS2 as well as MLH1, MSH2 and MSH6, and it is possible that this accounted for the higher sensitivity estimate.[54] Nevertheless, all sensitivity estimates were >80%. Due to a potential for spectrum bias, it would be expected that studies recruiting high-risk populations would result in higher sensitivity estimates than

those estimated from population-based studies. However, as discussed in a systematic review by Palomaki et al. (2009), and as with the MSI results reported above (*Section 2.2.5.1*) we did not find great differences between the sensitivity estimates in the population-based studies and the high-risk studies.[39] This could be because the three population-based studies with overall IHC data (Barnetson, 2006; Limburg, 2011; Southey, 2005) are based on age-limited populations and may also be subject to spectrum bias.[52-54]

For two of the population-based studies (Limburg, 2011; Southey, 2005) specificity, LR+, LR−, PPV and NPV estimates were also made for overall IHC results.[53, 54] These analyses were not conducted for the third population-based study (Barnetson, 2006) because overall IHC results were only available for reference standard positive participants.[52] Specificity was estimated as 91.9% (95% CI 86.3, 95.7) for Limburg et al. (2011) and 80.5% (95% CI 65.1, 91.2) for Southey (2005). LR+ was 10.6 (95% CI 5.7, 19.7) for Limburg (2011) and 5.1 (95% CI 2.8, 9.5) for Southey et al. (2005).[53, 54] LR− could only be estimated for one study (Limburg, 2011; 0.16 (95% CI 0.02, 0.95) because there were no false negative overall IHC results in Southey et al. (2005).[53, 54] PPV was lower in Limburg (2011) at 33.3% (95% CI 13.3, 59.0) than in Southey et al. (2005) at 69.2% (95% CI 48.2, 85.7). NPV was high in both studies: both estimates were >99%. Again, where apparent differences in IHC performance exist between these two studies (for example in PPV results) it should be considered that Southey et al. (2005) included an assessment of PMS2 in their results whereas Limburg et al. (2011) did not.[53, 54] Additionally, the specific techniques and methods used to perform the reference standard differ between studies and this may also impact upon apparent test performance.

Secondary overall IHC analyses were conducted where unclassified variants were considered to indicate a positive reference standard test result. Only two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary these analyses, and because Caldes et al. (2004) was based on a high-risk population and Hendriks (2003) is a reference standard positive study, only sensitivity estimates were made (75.0% [95% CI 57.8, 87.9] for Caldes, 2004; 88.6% [95% CI 76.0, 95.0] for Hendriks, 2003).[55, 59] For Caldes et al. (2004) this represents quite a reduction in sensitivity compared to when unclassified variants were considered to be index test negatives (96.4% [95% CI 81.7, 99.9]).[55]

Of the five study samples (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) that made an assessment of IHC for at least one individual protein, four studies provided data relevant to whether loss of expression in MLH1 was an accurate test result for assessing a pathogenic mutation in *MLH1*.[31, 52, 54, 59, 60] Three of these were population-based, single-gate studies (Barnetson, 2006; Southey, 2005; Poynter, 2008)[31, 52, 54] and one was a reference standard positive study (Hendriks, 2003).[59] The studies by Barnetson et al. (2006), Southey et al. (2005) and Hendriks et al. (2003) all provided data from which sensitivities were generated.[52, 54, 59] These ranged from 50.0% (95% CI 26.0, 74.0) for Southey et al. (2005) to 100.0% (95% CI 73.5, 100.0) for Barnetson et al. (2006).[52, 54] The three population-based studies (Barnetson, 2006; Southey, 2005; Poynter, 2008) provided data from which specificities were generated.[31, 52, 54] These ranged from 70.6% (95% CI 66.8, 74.2) for Poynter et al. (2008) to 96.0% (95% CI 93.1, 97.9) for Barnetson et al. (2006). The results for MSH2 were even more variable; three studies provided data for MSH2 (Barnetson, 2006; Southey, 2005; Hendriks, 2003) and sensitivities ranged from 22.2% (95% CI 6.4, 47.6) for Southey et al. (2005) to 81.8% (95% CI 48.2, 97.7) for Barnetson et al. (2006) and Hendriks et al. (2003).[52, 54, 59] The two population-based

studies (Barnetson, 2006; Southey, 2005) provided data from which specificities were generated with both being >92%.[52, 54] Four studies provided data for MSH6 and again, there was more variation in the sensitivities generated than in the specificities: sensitivities ranged from 44.4% (95% CI 21.5, 69.2) for Southey et al. (2005) to 75.0% (95% CI 19.4, 99.4) for Barnetson et al. (2006) and Hendriks et al. (2003), whereas specificities, which were only generated for the population-based studies (Barnetson, 2006; Southey, 2005) were >92% in both studies.[52, 54, 59] It was clear that, for loss of expression of MLH1, MSH2 and MSH6, the sensitivity estimates generated from Southey et al. (2005) were lower than those for the other studies.[54] It is possible that this is due to specific between-study differences in the assessment of IHC (a positive IHC result is a somewhat subjective judgement, made by human assessors, so interrater variability may impact upon results). Again, it is also possible that between-study differences in the reference standard could, to some extent, account for these differences in sensitivity estimates. Only the study by Southey et al. (2005) provided IHC data for PMS2, providing a sensitivity estimate of 55.6% (95% CI 30.8, 78.5) and a specificity estimate of 87.8 (95% CI 73.8, 95.9).[54]

LR+, LR−, PPV and NPV were calculated for the two population-based studies with sufficient available IHC data by protein (Barnetson, 2006; Southey, 2005).[52, 54] For MLH1, MSH2 and MSH6, LR+ was greater in Barnetson et al. (2006) than in Southey (2005): for MLH1, LR+ was 25.0 (95% CI 14.4, 43.5) for Barnetson et al. (2006) and 5.1 (95% CI 1.8, 14.5) for Southey et al. (2005); for MSH2, LR+ was 21.6 (95% CI 11.6, 40.2) for Barnetson et al. (2006) and 3.0 (95% CI 0.8, 12.2) for Southey (2005); for MSH6, LR+ was 16.2 (95% CI 7.6, 34.3) for Barnetson et al. (2006) and 6.1 (95% CI 1.8, 20.3) for Southey et al. (2005).[52, 54] For PMS2, data were only available from Southey et al. (2005), and LR+ was estimated as 4.6 (95% CI 1.8, 11.4).[54] There are several possible reasons why LR+ estimates were higher in Barnetson et al. (2006) than in Southey et al. (2005) including the fact that Barnetson et al. (2006) was a larger study than Southey et al. (2005), that the reference standard was not identical in these studies, and that there is a possibility that IHC ratings may have differed across studies (interrater reliability). For MLH1, LR− was only estimated for one study (Southey, 2005; 0.6, 95% CI 0.4, 0.9) because there were no false negative results in Barnetson et al. (2006). For MSH2, LR− was 0.2 (95% CI 0.1, 0.7) for Barnetson et al. (2006) and 0.8 (95% CI 0.7, 1.1) for Southey et al. (2005) and for MSH6, LR− was 0.3 (95% CI 0.1, 1.4) for Barnetson et al. (2006) and 0.6 (95% CI 0.4, 0.9) for Southey et al. (2005).[52, 54] For loss of protein expression in PMS2, data were only available from Southey et al. (2005), and LR− was estimated as 0.5 (95% CI 0.3, 0.9). PPV and NPV for MLH1 and MSH2 were largely consistent across the two studies (Barnetson, 2006; Southey, 2005).[52, 54] For MSH6, NPV estimates were consistent across the two studies, but PPV estimates were vastly different, with data from Barnetson et al. (2006) resulting in a PPV of 16.7 (95% CI 3.6, 41.4) and data from Southey et al. (2005) resulting in a PPV of 72.7 (95% CI 39.0, 94.0).[52, 54] Although the reason for this difference is not completely clear, it is likely due, at least in part, to the very low number of true positive results (n=3) for loss of expression in MSH6 in the study by Barnetson et al. (2006).[52] Again, only Southey (2005) provided data for PMS2, and PPV was estimated as 66.7 (95% CI 38.4, 88.2) and NPV as 81.8 (95% CI 67.3, 91.8).[54]

Secondary IHC analyses, for individual proteins, were conducted where unclassified variants were considered to indicate a positive reference standard test result. However, only one study (Hendriks, 2003) provided sufficient data to conduct secondary analyses and because this study is a reference standard positive study, only sensitivity estimates were made.[59]

These sensitivity estimates were very similar to those estimated from data where unclassified variants were considered to be reference standard negatives.

3 Assessment of end-to-end studies

End-to-end studies have an important place in HTAs of tests. They are broadly defined in the methods guidance as, “studies that follow patients from testing, through treatment, to final outcomes”. Such studies can have a wide variety of designs, but where available RCTs are noted to be of great importance because they provide comparative evidence with high internal validity. If end-to-end studies are found it may avoid the need for modelling as the end-to-end studies provide a direct linkage between a testing strategy and patient outcome, which otherwise could only be achieved by linkage in an economic model. For these reasons we specifically performed a systematic review of end-to-end studies, with a particular focus on RCTs and controlled clinical trials, recognising a priori that such studies were unlikely to exist for an intervention as complex as screening for Lynch syndrome.

3.1 Methods for reviewing effectiveness

3.1.1 Identification of studies

The same search was performed as for the review of diagnostic accuracy studies. This was appropriate as there were no restrictions by study design. In the protocol[67] we did not rule out the use of a methods filter to focus the search on intervention studies. However, this was not used in order to maximise the sensitivity of the searches.

3.1.2 Inclusion and exclusion criteria

The full inclusion criteria were as indicated in *Table 20* below. Concerning study design we agreed to consider other study designs if no includable RCTs or CCTs were identified.

Screening was performed by one primary screener (CH) and a 10% random sample checked by second reviewer (TS). In the screen, any study which appeared to investigate the impact of introducing the tests of interest on the outcomes of interest was retrieved in full text irrespective of the apparent study design or whether it appeared to be an abstract. In total, 3,920 citations were screened.

Twenty-two articles were retrieved in full text (details can be obtained from the authors on request). These reported 20 studies, as two articles were duplicate publications (both abstracts subsequently published in full). The final inclusion/exclusion decisions and abstraction of brief details about all articles retrieved in hard text was performed by a single reviewer (CH).

Further aspects of the method including data abstraction strategy, critical appraisal strategy and methods of data synthesis are not reported as there were no included studies.

Table 20: Inclusion and exclusion criteria for review of end-to-end studies

Criteria	Include	Exclude
Participants	• Studies of unselected or randomly selected CRC patients **OR** • CRC patients selected according to an age limit	• Studies using retained samples where storage methods may adversely affect test accuracy

Index tests	• Molecular MSI testing (with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing), including studies where *BRAF* V600E and/or *MLH1* methylation tests are performed according to MSI test results, followed by constitutional MMR mutation testing as described below*
	• MMR IHC (with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing), followed by the reference standard, followed by constitutional MMR mutation testing as described below*
	
	*Constitutional MMR mutation testing:
	
	• Including DNA sequencing, as a minimum, applied either to:
	  • All participants
	  • All participants testing positive for one or more index test and to a representative sample of patients testing negative for all index tests, but only if participants are a representative (not high-risk) sample of CRC patients
	AND
	• *MLH1*, *MSH2* and *MSH6* testing as a minimum (unless IHC results direct otherwise, or unless the aim of a study is to investigate the test accuracy of an index test in individuals with mutations in a particular MMR gene)
	AND
	• MLPA (including where only conducted when sequencing finds no clearly pathogenic mutations) or another technique for detecting large genomic abnormalities
		• Studies which do not include either of the index tests
		• Studies where mutation testing is limited to seeking only founder mutations

Comparators	Index tests may be compared with: • Each other • No testing for LS • Direct constitutional MMR mutation testing as described above*	

Outcomes	• Number of individuals receiving MSI and/or IHC testing • Number of individuals receiving subsequent tumour-based tests • Number of individuals receiving constitutional MMR mutation testing • Number of cascade tests on relatives • Number of Lynch syndrome diagnoses • Number of colonoscopies • Morbidity, mortality and/or life expectancy • Costs associated with interventions and comparators • Health-related quality of life	
Study design	• RCTs • CCTs	• Non-experimental, preclinical and animal studies and studies published *only* in abstract form • Systematic reviews of RCTS or CCTs

3.2 Results

3.2.1 Quantity and quality of research available

Eleven studies were excluded through failure to meet the first inclusion criterion applied, introduction of the index tests of interest. The remaining nine studies were all abstracts, and so excluded because they provided insufficient information to confirm the detailed nature of the testing introduced or to perform proper quality assessment of the study.

Even if the criterion about publication in full had been relaxed, only three studies could have been considered for inclusion, because six of the abstracts appeared to have no comparator. The three abstracts which did have a comparator were all pre-post studies measuring changes surrounding the introduction of testing for Lynch syndrome. None provided any contact details, and so it was not possible to easily enquire whether further information or full publication was available.

Two RCTs were identified amongst the hard text retrieved.

The first was a protocol from an RCT comparing Whole Exome Sequencing with “current practice” in screening for Lynch syndrome.[68]

The second was a cluster RCT investigating the effect of quality improvement initiatives.[69] The effect of quality improvement was further examined in a time-series analysis.[70]

Only one study assessed patient survival.

This examined whether conclusive follow-up testing, or not, following initial screen positive IHC testing in CRC cases in a specific institution was associated with better outcome. Thus it did not directly examine the effect of the introduction of screening for Lynch syndrome.[71] Further this study was only presented as an abstract and had limited contact details.

3.2.2 Assessment of effectiveness

3.2.2.1 Critical review and synthesis of information

We were not able to draw any conclusions on the effectiveness of screening for Lynch syndrome from the systematic review of end-to-end studies.

3.3 Discussion

Although the review identified some interesting near miss excluded studies providing evidence of indirect relevance to other aspects of the appraisal, there were no sufficiently relevant end-to-end studies reported in sufficient quality to obviate the need for a linked evidence approach using modelling. The three closest studies to inclusion were only reported as abstracts and it may be that full details would have revealed them to be excluded rather confirming their inclusion. All three employed pre-post designs and were hence highly susceptible to bias.

The review reinforces the lack of usefulness of studies which are only reported in abstract, further compounded by the fact that few abstracts provide any means to contact authors for further information. The review also invites consideration of whether some study designs which might currently be considered as end-to-end studies, such as pre-post studies, may be too open to bias to be worth including in a systematic review of end-to-end studies, even

if they are the only evidence available. This does not preclude them being used to parameterise a model, provided the openness to bias is fully acknowledged.

We believe the method of the review, particularly the extremely comprehensive search, which was not restricted by a study design filter as originally planned, makes it unlikely that we have missed major items of published literature. It is possible that we may have overlooked unpublished literature, as searching for this is extremely difficult to achieve in short time-scales. We did however fully consider conference abstracts appearing in the main bibliographic databases we included in our search, although they did not yield useful information for reasons already indicated. Ideally we could have used double screening and in/exclusion in duplicate. This did not seem to be justified for screening after good agreement was achieved in the 10% random check of screening decisions. Similarly although not formally checked we are confident that the limited number of decisions on inclusion/exclusion, performed by an experienced reviewer using a well-developed set of inclusion/exclusion criteria was accurately performed. We did not feel checking this review step was a priority amongst the other tasks required to complete the HTA.

4 Assessment of existing cost-effectiveness evidence

The cost-effectiveness of using microsatellite instability (MSI) and immunohistochemistry (IHC) in strategies to identify Lynch syndrome was assessed by conducting a systematic review of published research evidence.

4.1 Objectives

The objectives of this systematic review were to:

- Gain insights into the key drivers of cost-effectiveness in this disease area;
- Get an overview of the alternative modelling approaches that have been adopted in this disease and treatment area;
- Provide a summary of the findings of previous relevant cost-utility, cost-effectiveness, and cost-benefit studies generalisable to the UK.

4.2 Methods

4.2.1 Study identification

This systematic review was an update of the systematic review of cost-effectiveness reported in Snowsill et al. (2014).

The search strategy included the following sources:

- Searching of electronic databases
    - MEDLINE (Ovid)
    - MEDLINE In-Process & Other Non-Indexed Citations (Ovid)
    - Embase (Ovid)
    - Web of Science (Thomson Reuters)
    - NHS EED (The Cochrane Library)
    - EconLit (EBSCO)
- The reviews by Snowsill et al.[4] and Grosse[72]
- Backward citation chasing on included studies

The searches were developed and run by an information specialist (SB) in February 2016. Search filters were used to limit the searches to economic or health utilities studies as appropriate, and searches were limited to English language studies where possible. A date limit of 2013 was used. The search strategies for each database are detailed in *Appendix 1*.

The database search results were exported to, and de-duplicated using, Endnote (X7). De-duplication was also performed using manual checking. After the reviewers completed the screening process, the bibliographies of included papers were scrutinised for further potentially includable studies.

Titles and abstracts returned by the search strategy were examined independently by two researcher (NH, TS) and screened for possible inclusion. Disagreements were resolved by

discussion. Full texts of potentially relevant studies were ordered. Full publications were assessed by the same reviewers (NH, TS) for inclusion or exclusion against pre-specified criteria. Again disagreements were resolved by discussion.

Reviewers also examined the included studies of the systematic reviews by Snowsill et al. (2014) and Grosse (2015)[72] for other potential includes.

4.2.2 Eligibility criteria

Table 21 shows the inclusion criteria for the systematic review of published cost-effectiveness studies, compared to the inclusion criteria for the previous PenTAG review. The inclusion criteria for the current review are narrowed to address the decision problem, meaning that searches only need to be run for dates after the previous PenTAG review.

In the protocol for this project it is stated that IHC should be treated as a comparator only and not an intervention. We have updated the inclusion criteria for this review, so that IHC is now treated as an intervention. This means that included studies which contain IHC-based strategies do not need to also contain MSI-based strategies, as specified when IHC is treated as a comparator. This also ensures consistency between the clinical and cost-effectiveness reviews.

Systematic reviews, if identified, were not directly included, but their bibliographies were searched for potentially includable studies.

Table 21: Cost-effectiveness inclusion criteria

PICOS criteria	Previous PenTAG review[4]	Current review
Population	Persons who may or may not have Lynch syndrome	All people newly diagnosed with colorectal cancer
Intervention	Any of the following (including combinations): • Strategies to identify Lynch syndrome in the population • Strategies to manage Lynch syndrome in the population • Strategies to manage patients in whom Lynch syndrome is identified	One of • Microsatellite instability testing (with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing) • Immunohistochemistry (with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing)
Comparator	Current clinical practice (may or may not include efforts to identify Lynch syndrome)	At least one of: • The included interventions • No testing • Direct constitutional MMR mutation testing
Outcomes	Any of the following: • Costs • Clinically relevant outcomes (e.g., life-years gained, QALYs, CRCs prevented) • Mutations detected	Costs and health effects measured in life years or QALYs
Study type	Any of the following: • Decision-analytic models (with or without a cost-effectiveness component) • Evaluations of cost-effectiveness within	Any of the following: • Decision analytic models • Economic evaluations within trials • Cost or resource use studies from the UK

	trials (including cost-effectiveness, cost-utility and cost-benefit studies; no requirement for randomisation) • Cost or resource use studies • Guidelines from national institutions, professional bodies and international bodies (including working groups)

Key: CRC, colorectal cancer, MMR, mismatch repair; QALY, quality adjusted life year

4.2.3 Data extraction

Data was extracted by one reviewer (NH), using the blank data extraction forms used in the previous PenTAG review.

The completed forms for studies included in the previous PenTAG review were reused.

The evidence base was assessed using narrative synthesis supported by summary data extraction tables, constructed for the previous PenTAG review. Where studies do not conduct a fully incremental cost-effectiveness analysis (e.g., if they perform a cost–consequences analysis), but it was possible to conduct such an analysis based on reported results, this was done.

Currency conversion was not performed, but an indication of purchasing-power-parity exchange rates was given, and if currency- or country-specific cost-effectiveness thresholds were supplied by the authors these were also reported (in the original currency).

4.2.4 Critical appraisal

To remain consistent with the previous PenTAG cost-effectiveness review, the quality appraisal was conducted using selected criteria from the Drummond checklist. Quality appraisal was conducted by one reviewer (NH).

Additionally, a set of review-specific criteria was developed for the previous PenTAG review, and this was adapted to reflect the current decision problem.

4.3 Results

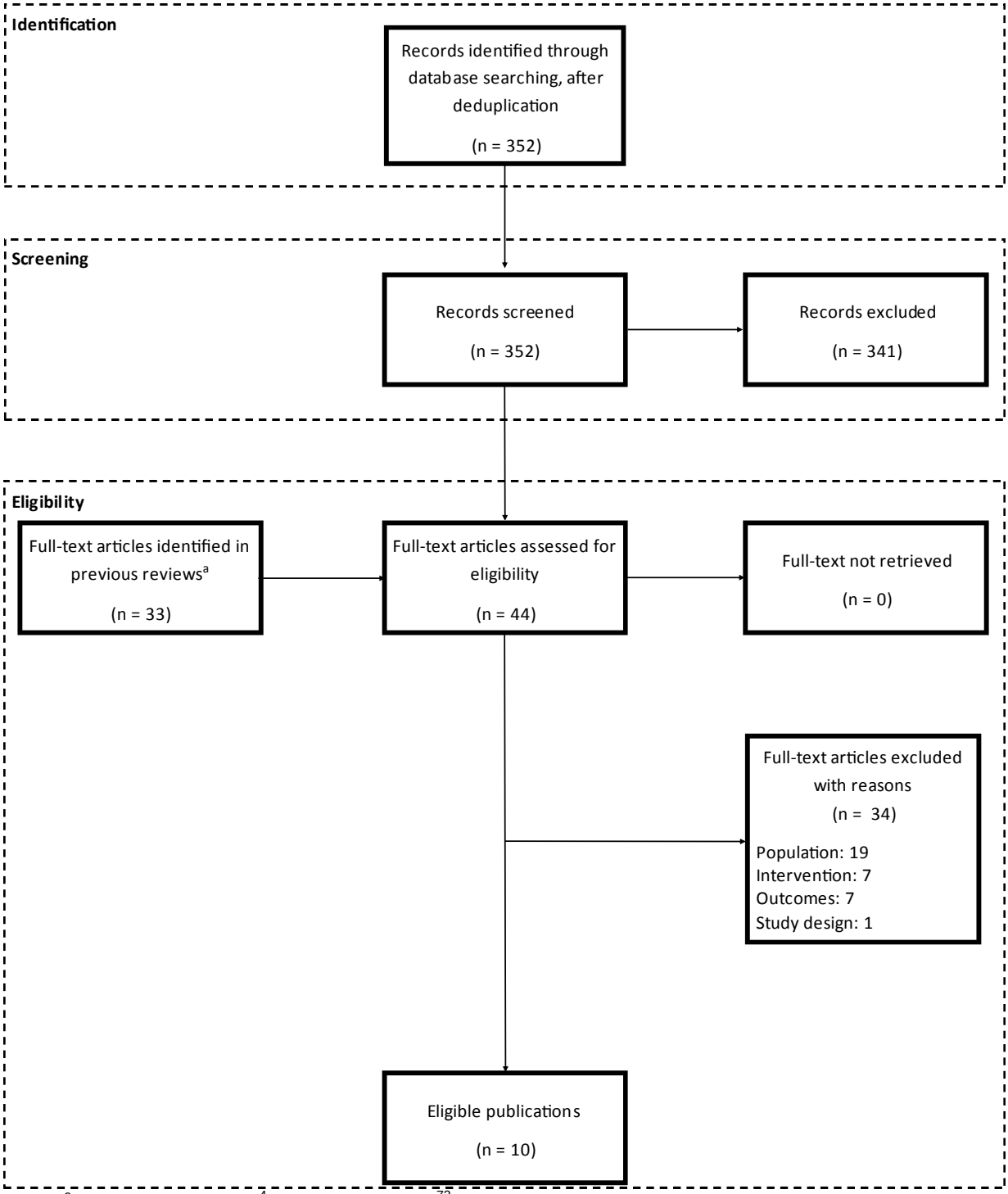
Figure 6 shows the study flow diagram of this update review. The electronic database search for cost-effectiveness evidence identified 352 records after deduplication. All were screened by title and abstract. Of these 11 were identified for full-text screening. All 11 full texts were retrieved and assessed for eligibility.

Of the 11 full texts assessed for eligibility, six were deemed to meet the eligibility criteria.

Of the 33 additional publications identified in previous reviews,[4, 72] six were deemed to meet the eligibility criteria for this review and were assessed in full. The remaining 29 were excluded on the basis of population, intervention or outcome.

One UK based cost study was identified,[73] but in this study the population was identified using clinical criteria and was therefore ineligible for inclusion in our review.

Figure 6: PRISMA flow diagram for cost-effectiveness papers.



Notes: [a] Snowsill et al. (2014)[4] and Grosse (2015)[72]

4.3.1 Characteristics of identified cost-effectiveness studies

The characteristics of the 10 included papers and their results are given in *Table 22* and *Table 23*. Some studies report results of strategies that use clinical criteria to identify Lynch syndrome. Where possible, these results are excluded. We note that one study is reported in abstract.[74] One study is reported in two separate papers (Snowsill et al. 2014, and Snowsill et al. 2015).[4, 75] These two papers have been grouped together for reporting purposes, giving a total of 9 separate studies.

The majority of studies (7 out of 9) report results for a US population.[74, 76-81] Of the other two studies, one is from the perspective of a German population[82] and one is UK based.[4, 75]

All 9 studies include strategies to identify Lynch syndrome in index CRC patients (probands) and relatives of CRC patients. The majority of studies identify relatives through cascade testing, but two studies identify only first degree relatives (FDRs) of the index CRC patient and in two other studies the method of identifying relatives is unclear.

Where reported, the modelling approach appears similar across studies. Models are split into two sections: a diagnostic model to identify Lynch syndrome (often a decision tree); and a long term model to estimate the costs and benefits associated with the outcomes of the diagnostic model.

All studies included either IHC or MSI based diagnostic strategies and a universal testing strategy as a comparator. Six studies[4, 75, 77-80, 82] included both IHC and MSI based strategies allowing for a comparison between the two interventions. The optimal strategy varied across these studies and depended greatly on the willingness to pay threshold of the relevant country, or the main relevant comparator, so no strategy was consistently seen to be the most cost-effective.

Lynch syndrome status was confirmed with germline testing of *MLH1*, *MSH2*, *MSH6* and *PMS2* in all studies. Gallego et al. (2015) considered the use of next generation sequencing (NGS).[81] However, instead of comparing the cost-effectiveness of strategies to diagnose Lynch syndrome, Gallego et al. (2015) investigated the cost-effectiveness of using NGS to identify a wider range of hereditary colorectal cancer predispositions, which included Lynch syndrome. Therefore this analysis did not include a strategy without testing

All studies modelled the benefit of colonoscopic surveillance for Lynch syndrome positive relatives, with most (6 studies) also explicitly offering the same surveillance to probands.[4, 75, 76, 78-81] The frequency of this surveillance did change across studies, but was usually modelled on an annual or biannual interval, based on recommended guidelines.

Four studies also included prevention for gynaecological cancers (prophylactic hysterectomy and bilateral salpingo-oophorectomy [H-BSO] or gynaecological screening).[4, 75, 78-80] Barzi et al. (2015), Ladabaum et al. (2011) and Wang et al. (2012) all included both endometrial cancer and ovarian cancer, whereas Snowsill et al. (2014, 2015) only included endometrial cancer. Ladabaum et al. and Wang et al. were the only studies to include gynaecological screening and in their base cases, only a cost was applied for this screening; no benefit was assumed.

One study considered the use of aspirin prevention.[82] Severin et al. (2015) used data from the CAPP2 study to apply a hazard ratio of 63% for a maximum of 11 years (observation time of CAPP2) to reduce the incidence of CRC in patients taking daily aspirin. We discuss this trial in more detail in *Section 5.1.4.1.4*, *page 190*). Both the cost of treatment with aspirin

and the costs of complication from aspirin were included at a total annual cost of €596 (2012 costs).

Of the nine cost-effectiveness studies, five were cost-utility studies. These are the five studies we focus on for the rest of the review.

Table 22: Study characteristics

Author, year published	Setting, perspective	Population	Study purpose	Study approach	Diagnostic comparators	Treatment strategies
Ramsey, 2003[76]	USA, US healthcare system	Newly diagnosed CRC patients and their siblings and children	Cost-effectiveness of strategies to identify LS	Decision model Ad hoc	Probands Test 1 / Test2 / Test3 None Bethesda / MSI / GT MSI / GT Bethesda / GT GT Bethesda: Revised Bethesda Criteria Relatives (proband confirmed mutation/ indeterminate result) Germline testing for siblings and children	Confirmed mutation LS colorectal surveillance and prophylactic surgery on CRC diagnosis Indeterminate result LS colorectal surveillance LS negative Probands- standard care Relatives- no further action
Mvundura, 2010[77]	USA, US healthcare system	Newly diagnosed CRC patients and relatives	Cost-effectiveness and cost-utility of strategies to identify LS	Decision model Ad hoc	Probands Test1 / Test 2 / Test 3 None IHC / *BRAF* (abnormal IHC for MLH1) / GT (MLH1) IHC / GT (all other abnormal IHC) IHC / GT MSI / GT GT Relatives (proband confirmed mutation) GT	Relatives with confirmed mutation: Colonoscopy every 1-2 years from age 20-25 until 79 LS negative relatives (and some positive who declined LS surveillance): Colonoscopy every 10 years from age 50

Author, year published	Setting, perspective	Population	Study purpose	Study approach	Diagnostic comparators	Treatment strategies
Ladabaum, 2011[78]	USA, Third-party payer (NIH)	Newly diagnosed CRC patients and relatives	Cost-effectiveness of strategies to identify LS	Decision model Decision tree model, Markov subtrees	Probands Test 1 / Test2 / Test3 None CC / GT CC / IHC / GT TBT / GT GT Relatives (proband confirmed mutation) Germline testing	Persons with confirmed LS mutations or assumed LS and their FDRs: Annual colonoscopy from age 25 Women-gynaecological screening from age 35, prophylactic TAH-BSO at age 40 Others: Colonoscopy every 10 years from age 50
Wang, 2012[79]	USA, Third-party payer (NIH)	Newly diagnosed CRC patients and relatives	Cost-utility of strategies to identify LS (update of Ladabaum to include QoL)	Decision model Decision tree model, Markov subtrees	Probands Test 1 / Test2 / Test3 None CC / GT CC / IHC / GT TBT / GT GT Relatives (proband confirmed mutation) Germline testing	Persons with confirmed LS mutations or assumed LS and their FDRs: Annual colonoscopy from age 25 Women-gynaecological screening from age 35, prophylactic TAH-BSO at age 40 Others: Colonoscopy every 10 years from age 50
Gallego, 2014[74] (abstract)	USA, NR	CRC patients and relatives	Cost-effectiveness and cost-utility of strategies to identify LS	Decision model	Probands Test 1 / Test2 IHC/MSI / Targeted sequencing IHC/MSI / NGS NGS Relatives NR	Relatives with LS detected: CRC surveillance

<table>
<tr><th>Author, year published</th><th>Setting, perspective</th><th>Population</th><th>Study purpose</th><th>Study approach</th><th>Diagnostic comparators</th><th>Treatment strategies</th></tr>
<tr><td>Snowsill, 2014[4]
Snowsill, 2015[75]</td><td>UK, NHS & PSS</td><td>Newly diagnosed CRC patients <50 years old and relatives</td><td>Cost-utility of strategies to identify LS</td><td>Decision model
Decision tree model and an individual patient simulation model</td><td>Probands
<table><tr><td>Test 1</td><td>Test2</td><td>Test3</td><td>Test 4</td></tr><tr><td colspan="4">None</td></tr><tr><td colspan="4">ACII</td></tr><tr><td>IHC</td><td colspan="3">GT</td></tr><tr><td>IHC</td><td>BRAF</td><td colspan="2">GT</td></tr><tr><td>MSI</td><td colspan="3">GT</td></tr><tr><td>MSI</td><td>BRAF</td><td colspan="2">GT</td></tr><tr><td>MSI</td><td>BRAF</td><td>IHC</td><td>GT</td></tr><tr><td>IHC</td><td>MSI</td><td>BRAF</td><td>GT</td></tr><tr><td colspan="4">GT</td></tr></table>
Relatives
Proband confirmed LS: genetic testing
Proband suspected LS: FDRs assumed to have LS</td><td>Biannual colonoscopic surveillance for LS positive/suspected probands and relatives (from age 25)

Prophylactic TAHBSO offered to women with LS diagnosis at 45 years old</td></tr>
<tr><td>Barzi, 2015[80]</td><td>US, societal</td><td>CRC patients and their relatives and general population</td><td>Cost-effectiveness of strategies to identify LS</td><td>Decision model
Decision tree and individual level microsimulation Markov model</td><td>Probands
<table><tr><td>Test 1</td><td>Test2</td><td>Test3</td><td>Test 4</td></tr><tr><td colspan="4">None</td></tr><tr><td>CC</td><td colspan="3">GT</td></tr><tr><td>CC</td><td>IHC</td><td colspan="2">GT</td></tr><tr><td>IHC</td><td colspan="3">GT</td></tr><tr><td>IHC+BRAF</td><td colspan="3">GT</td></tr><tr><td>MSI</td><td colspan="3">GT</td></tr><tr><td>MSI+IHC</td><td colspan="3">GT</td></tr><tr><td>MSI+IHC+BRAF</td><td colspan="3">GT</td></tr><tr><td colspan="4">GT</td></tr></table>
Relatives
FDRs GT

General population screening
PREMM >20y, >25y, >30y, >35y</td><td>Annual colonoscopy age 20-80

TAH-BSO offered at age 40</td></tr>
</table>

Author, year published	Setting, perspective	Population	Study purpose	Study approach	Diagnostic comparators	Treatment strategies
Gallego, 2015[81]	US	CRC patients and relatives	Cost-effectiveness and cost-utility of NGS in strategies to identify CRCP (including LS)	Decision model Decision tree Long term estimates based of Mvundura et al. (2010) results	Probands Standard care: IHC, *BRAF*, GT Intervention: NGS panels. For our purposes panel 1- LS only Relatives GT	Surveillance for patients and relatives
Severin, 2015[82]	Germany, German Statutory Health Insurance system	Newly diagnosed CRC patients and FDRs	Cost-effectiveness of strategies to identify LS	Decision model Decision tree plus Markov model	(FH) IHC *BRAF* GT (FH) IHC MSI GT (two strategies, MSI depending on IHC outcome) (FH) MSI IHC GT (FH) MSI GT (FH) GT	LS relatives Annual colonoscopy Aspirin chemoprevention LS negative/unknown relatives Colonoscopy every 10 years between 55 and 75

Key: CC[1], clinical criteria or prediction model (Amsterdam Criteria II, Revised Bethesda, PREMM, MMRPro, MMRpredict); TBT, Tumour based test (MSI, IHC, IHC + *BRAF*, MSI with IHC, MSI with IHC + *BRAF*); GT, genetic testing; IHC, immunohistochemistry; *BRAF*, *BRAF* V600E test; PREMM, PREMM prediction model; MSI, microsatellite instability; NGS, next generation sequencing; NR, not reported; FDR, first degree relative; LS, Lynch syndrome

Table 23: Study results

Author, year published	Outcomes measured	Discount rate	Base results	Sensitivity analysis approach	Main sensitivity analysis results
Ramsey, 2003[76]	Life years Costs ICERs	3%	Compared to no screening, all strategies cost-effective ($50,000 threshold) apart from universal testing. ICERs: Strategy: ICER ($/LY) Bethesda/MSI: 11,865 MSI: 394,067 Bethesda: 441,172 Universal: 2,553,345	Univariate analysis PSA	*Univariate* Sensitive to survival benefit for increased surveillance in LS +ve, specificity of family history/ MSI, prevalence of LS in probands, *PSA* Unclear which strategy is most cost effective, but universal testing is the least cost effective.
Mvundura, 2010[77]	Life years Costs ICERs *QALYs*	3%	All strategies with preliminary tests cost-effective relative to no testing. IHC and *BRAF* as preliminary were optimal (ICER $22,552/LY)	Univariate sensitivity analysis Scenario analyses	*Univariate* Most sensitive to CRC risk among relatives, number of relatives per proband and compliance to surveillance. *Scenario analyses* Using median lab prices increased costs and meant age-targeted IHC and *BRAF* was most cost effective. Cascade testing reduced all ICERs relative to no testing. Using QALYs on average scaled ICERs by 1.18 LY/QALY
Ladabaum, 2011[78]	Life years Costs ICERs *Cancer cases* *Cancer deaths*	3%	**Strategy** / **ICER ($/LY)** / **ICER excl. CC strategies** MMRpro/IHC: 30,600: - Bethesda/IHC: 39,600: - MMRpro: 41,400: - Bethesda: 50,200: - IHC (+*BRAF*): -: 36,200 MSI + IHC (+*BRAF*): 117,000: 108,000 Universal GT: 293,000: 293,000 All other strategies dominated or extended dominated	Univariate sensitivity analysis PSA Scenario analyses	*Univariate* Most sensitive to age of relative, effectiveness of LS surveillance for CRC and prevalence of LS. *PSA* IHC(+*BRAF*) optimal strategy in 53% of iterations. *Scenario analyses* Age limit for probands improves cost-effectiveness. 3-4 relatives needed for most strategies to be cost-effective compared to doing nothing.

<table>
<tr><th>Author, year published</th><th>Outcomes measured</th><th>Discount rate</th><th>Base results</th><th>Sensitivity analysis approach</th><th>Main sensitivity analysis results</th></tr>
<tr>
<td>Wang, 2012[79]</td>
<td>QALYs
Costs
ICERs</td>
<td>3%</td>
<td>
<table>
<tr><th>Strategy</th><th>ICER ($/QALY)</th><th>ICER excl. CC strategies</th></tr>
<tr><td>MMRpro/IHC</td><td>50,562</td><td>-</td></tr>
<tr><td>Bethesda/IHC</td><td>65,347</td><td>-</td></tr>
<tr><td>MMRpro</td><td>68,384</td><td>-</td></tr>
<tr><td>Bethesda</td><td>82,864</td><td>-</td></tr>
<tr><td>IHC (+BRAF)</td><td>-</td><td>59,719</td></tr>
<tr><td>MSI + IHC (+BRAF)</td><td>193,343</td><td>179,576</td></tr>
<tr><td>Universal GT</td><td>393,303</td><td>271,219</td></tr>
</table>
All other strategies dominated or extended dominated
</td>
<td>Univariate sensitivity analysis

PSA

Scenario analyses</td>
<td>Univariate
Results consistent with Ladabaum et al. 2011

PSA
IQRs narrow and within cost-effective ranges, ICERs had wide 95% CIs, reflecting wide distributions of utility estimates.

Scenario analyses
Length of effect from disutility associated with GT or surveillance affected ICERs: longer than 12 months and the ICERs exponentially increased.</td>
</tr>
<tr>
<td>Gallego, 2014[74]</td>
<td>Life years
QALYs
Costs
ICERs</td>
<td>NR</td>
<td>Universal NGS testing vs. reference strategy $196,000 per QALY gained
IHC/MSI followed by NGS $71,000 per QALY gained</td>
<td>Univariate sensitivity analysis</td>
<td>Most influential parameters: number of relatives tested, prevalence of LS in CRC, cost of CRC surveillance in relatives.</td>
</tr>
</table>

<table>
<tr><th>Author, year published</th><th>Outcomes measured</th><th>Discount rate</th><th>Base results</th><th>Sensitivity analysis approach</th><th>Main sensitivity analysis results</th></tr>
<tr>
<td>Snowsill, 2014[4]
Snowsill, 2015[75]</td>
<td>Life years
QALYs
Costs
ICERs
INHB</td>
<td>3.5%</td>
<td>
<table>
<tr><th>Strategy</th><th>ICER vs no testing (£/QALY)</th><th>ICER (£/QALY)</th></tr>
<tr><td>No testing</td><td>-</td><td>-</td></tr>
<tr><td>ACII</td><td>6,021</td><td>Extended dominated</td></tr>
<tr><td>IHC GT</td><td>6,444</td><td>Dominated</td></tr>
<tr><td>IHC BRAF GT</td><td>5,831</td><td>Extended dominated</td></tr>
<tr><td>MSI GT</td><td>5,610</td><td>Dominated</td></tr>
<tr><td>MSI BRAF GT</td><td>5,491</td><td>5,491</td></tr>
<tr><td>MSI BRAF IHC GT</td><td>5,774</td><td>Dominated</td></tr>
<tr><td>IHC MSI BRAF GT</td><td>7,601</td><td>25,106</td></tr>
<tr><td>Universal GT</td><td>9,571</td><td>82,962</td></tr>
</table>
</td>
<td>Scenario analyses

Univariate sensitivity analyses</td>
<td>Scenario analysis:

EC excluded
Cost-effectiveness of strategies improve. MSI BRAF GT strategy still most cost-effective £4,439 per QALY gained

BRAF replaced by methylation
MSI GT strategy most cost-effective £7,965 per QALY gained (vs. MSI BRAF GT)

Age limit increased
Age 60
MSI BRAF GT most cost-eff £7,681 per QALY gained
Age 70
MSI BRAF GT most cost-effective £10,247 per QALY gained

Univariate sensitivity analyses
Most sensitive parameters were disutilities for EC and prophylactic TAHBSO. Other sensitive parameters included: #relatives, prevalence of LS, cost of colonoscopy complications, CRC incidence, effectiveness of colonoscopy to prevent CRC.</td>
</tr>
</table>

<table>
<tr><th>Author, year published</th><th>Outcomes measured</th><th>Discount rate</th><th>Base results</th><th>Sensitivity analysis approach</th><th>Main sensitivity analysis results</th></tr>
<tr><td>Barzi, 2015[80]</td><td>Life years
Costs
ICERs</td><td>3%</td><td>For scenario 3 (which includes costs and benefits for probands), ignoring CC strategies
IHC followed by GT most cost-effective strategy: ICER ~$50,000 per LYG versus no testing. Universal GT ~$131,000 per LYG vs. no testing, ~$943,000 per LYG vs. IHC->GT. All other tumour testing strategies dominated.</td><td>Scenario analysis

Univariate sensitivity analysis</td><td>Results were most sensitive to cost of germline testing.</td></tr>
<tr><td>Gallego, 2015[81]</td><td>Life years
QALYs
Costs
ICERs</td><td>3% (benefits NR)</td><td>NGS vs. standard care $144,235 per QALY gained</td><td>Scenario analyses

Univariate sensitivity analyses</td><td>NR for Panel 1 (our intervention of interest)</td></tr>
<tr><td>Severin, 2015[82]</td><td>Life years
Costs
ICERs</td><td>NR</td><td><table><tr><th>Strategy</th><th>ICERs (euro/LYG)</th></tr><tr><td colspan="2">No screening</td></tr><tr><td>Counselling including Bethesda, IHC, BRAF, sequencing</td><td>77,268</td></tr><tr><td>Counselling, IHC, BRAF, sequencing</td><td>253,258</td></tr><tr><td>Counselling, direct sequencing</td><td>4,188,036</td></tr></table></td><td>Scenario analyses

Univariate sensitivity analyses

PSA</td><td>PSA
€50,000 per LYG No screening 87% chance of being considered cost effective

Scenario analyses
Uptake of testing by FDRs influential on ICER

Aspirin has small impact on cost-effectiveness

Univariate
relatives, prevalence of LS.</td></tr>
</table>

Key: ACII, Amsterdam criteria II; *BRAF*, *BRAF* V600E test; CC, clinical criteria; CRC, colorectal cancer; FDR, first-degree relative; GT, genetic testing; ICER, incremental cost-effectiveness ratio; IHC, immunohistochemistry; LS, Lynch syndrome; LYG, life year gained; MSI, microsatellite instability; NGS, next generation sequencing; NR, not reported; PREMM, PREMM prediction model; PSA, probabilistic sensitivity analysis; QALY, quality adjusted life year; SA, sensitivity analysis

4.3.1.1 Mvundura et al. (2010)

Mvundura et al. (2010) produced a decision model of newly diagnosed CRC patients and their relatives in the USA. It compared IHC (with or without BRAF testing), MSI testing, no testing and universal genetic testing. Relatives were identified through genetic testing. Whilst the study did include both MSI and IHC testing strategies, it did not include all the interventions identified in the NICE Scope.

Relatives with a confirmed mutation were offered colonoscopy every 1-2 years from age 20-25 until 79. Lynch syndrome negative relatives and some of those who were Lynch syndrome positive and declined the intensive Lynch syndrome surveillance were offered colonoscopy every 10 years from age 50.

The analysis does not include Lynch related cancers other than CRC, or the differences in CRC incidence between males and females. It is also does not include all comparators identified in the NICE Scope

In their base case Mvundura et al. reported only life years gained (LYG), as opposed to quality-adjusted life years (QALYs). All strategies with preliminary tests were found to be cost-effective compared to no testing. IHC followed by *BRAF* testing was the optimal strategy with an ICER of $22,552 per LYG. To turn their analysis into a cost-utility analysis, the ICERs were scaled by a factor of 1.18 LY/QALY. This did not impact the order of the strategies and increased the ICERs for all strategies. This approach requires a number of assumptions to be made and therefore is unlikely to reflect the true cost per QALY gained for each testing strategy.

The model results were sensitive to CRC risk in relatives, number of relatives and adherence to surveillance.

4.3.1.2 Wang et al. (2012)

Wang et al. (2012) provide an update of Ladabaum et al. (2011), looking at the cost-utility of strategies to identify Lynch syndrome in newly diagnosed CRC patients and their relatives in the US. The difference between the two models was the inclusion of utilities in the update.

Wang et al. included strategies that started with clinical criteria (ACII and revised Bethesda or prediction models) as well as those that began with tumour based testing (IHC, MSI with our without *BRAF*). We do not focus on the strategies that begin with clinical criteria as these are not considered as part of our analysis. As with Mvundura et al., not all interventions identified by the NICE Scope were included.

The model used a decision tree with Markov subtrees to model newly diagnosed CRC patients and their relatives. For people identified with Lynch syndrome mutation, or with unconfirmed diagnosis of Lynch syndrome (plus their first degree relatives), annual colonoscopy (from age 25) was offered. Women were offered gynaecological screening from age 35 and prophylactic total abdominal H-BSO (TAH-BSO) from age 40. When Lynch syndrome was not diagnosed, people were offered 10-yearly colonoscopy from age 50.

The strategy which used IHC (with or without *BRAF* V600E mutation testing) was found to be the most cost-effective strategy, with an ICER of $59,719 per QALY gained. With the exception of the strategies of MSI in combination with IHC (with or without *BRAF* V600E testing) or universal germline testing (both of which had ICERs >$100,000 compared to the

next most cost-effective strategy), all other strategies were dominated or extended dominated.

The results were most sensitive to age of relative, effectiveness of Lynch syndrome surveillance for CRC and prevalence of Lynch syndrome.

4.3.1.3 Gallego et al. (2014)

Gallego et al. examines the cost-effectiveness and cost-utility of strategies to identify Lynch syndrome in CRC patients and their relatives. Details of the modelling are scarce as this is reported only as an abstract. The main comparison appears to be looking at targeted sequencing versus next generation sequencing. It is also unclear whether this study uses the same underlying model as that reported in Gallego et al. (2015), but the author list suggests the two are likely to be related. For relatives in whom Lynch syndrome is detected, CRC surveillance is offered, though again details are not given. The reference strategy was a combination of IHC and MSI followed by targeted sequencing. Replacing targeted sequencing with NGS gave an ICER of $71,000 per QALY gained and a strategy of NGS for all patients versus the reference strategy gave an ICER of $196,000 per QALY gained.

As with most other analyses the most influential parameters include number of relatives tested, prevalence of Lynch syndrome in CRC, cost of CRC surveillance in relatives.

4.3.1.4 Snowsill et al. (2014, 2015)

Snowsill et al. (2014 and 2015) report the previous work of PenTAG, which assessed the cost-effectiveness of strategies to diagnose Lynch syndrome in newly diagnosed colorectal cancer patients under 50 years old, and their relatives. Snowsill et al. presented a cost-utility analysis from the perspective of the NHS and PSSRU and included both CRC and endometrial cancer as outcomes. They stated that where possible they adhered to the NICE reference case. The model utilised a decision tree diagnostic section and an individual sampling model to calculate long term outcomes and survival for each of the diagnoses from the diagnostic section. Probands and relatives who received a confirmed (mutation positive) or assumed Lynch syndrome diagnoses (probands and relatives who were offered but declined germline testing and first degree relatives of probands who were assumed to have Lynch syndrome) were offered colonoscopic surveillance, and prophylactic TAH-BSO at age 40 if they were women.

In this analysis, all testing strategies had ICERs of less than £10,000 per QALY gained. When compared incrementally, MSI followed by *BRAF* testing was most cost-effective at a willingness to pay threshold of £20,000. Scenario analyses demonstrated that increasing the age of the population increased the ICERs for each strategy compared to no testing, though MSI plus *BRAF* remained the most cost-effective strategy. The model appeared most sensitive to the disutilities for EC and prophylactic TAHBSO. Other sensitive parameters included: number of relatives, the prevalence of Lynch syndrome, the cost of colonoscopy complications, the CRC incidence, and the effectiveness of colonoscopy to prevent CRC.

Though we believe this previous work was the first UK based model to assess the cost-utility of strategies to diagnose Lynch most complete models and has been described by Ladabaum et al. (2015) as the “most comprehensive decision model”[3], this model still does not fully answer the decision problem set out in this assessment. The strategies Snowsill et al. included are not in line with the current scope, with several strategies including a combination of MSI and IHC testing, and methylation testing was only included as a scenario

analysis, in place of *BRAF* V600E testing. The parameters are also now several years old, and were chosen for colorectal cancer patients under 50 years old, so they are unlikely to represent the overall population and the current clinical experience.

4.3.1.5 Gallego et al. (2015)

Gallego et al. present a cost-utility analysis of CRC patients and their relatives form a US perspective. It uses a decision tree model with long term outcome estimates based on results presented in Mvundura et al. (2010). As such the concerns we have with that cost utility analysis are equally present in this analysis.

In this study the strategies were IHC followed by *BRAF* prior to germline testing. The intervention strategies were next generation sequencing panels. The relevant intervention strategy for our analysis is Panel 1, which looked at Lynch syndrome only. CRC surveillance was offered for both probands and relatives, though details of this surveillance were not given. As Mvundura et al. (2010) is the stated to be the source of the long term outcomes, we assume that surveillance is therefore modelled as in Mvundura et al.

NGS for Lynch syndrome versus standard care gave an ICER of $144,235 per QALY gained. Sensitivity analyses were not reported for this scenario, as it was not the base case.

4.3.2 Quality of identified cost-effectiveness studies

Results of the quality appraisal are provided in *Table 24* and *Table 25*.

In general reporting ranged from mixed to quite good. Snowsill et al. appeared to be the most comprehensively reported. Very few studies reported endometrial cancer and only Barzi et al. (2015), Wang et al. (2012) and Ladabaum et al. (2011) included ovarian cancer as well.

Table 24: Selected criteria from Drummond checklist

Study	The viewpoint(s) of the analysis are clearly stated and justified	The source(s) of effectiveness estimates are stated	Methods for the estimation of quantities and unit costs are described	Currency and price date are recorded	Details of any models used are given	Time horizon of costs and benefits is stated	The ranges over which the variables are varied are justified
Ramsey, 2003	✗	✓	✓	✓	✗	✓	✓
Mvundura, 2010	✓	✓	✓	✓	✓	✓	✓
Ladabaum, 2011	✓	✓	✓	✓	✓	✓	✓
Wang, 2012	✗	✓	✓	✓	✓	✓	✓
Snowsill, 2014, 2015	✓	✓	✓	✓	✓	✓	✓
Barzi, 2015	✓	✓	✗	✗	✓	✓	✗
Gallego, 2015	✗	✓	✓	✗	✗	✗	✓
Severin, 2015	✓	✓	✓	✓	✓	✓	✓

Source: Drummond and Jefferson 1996[83]

Table 25: Selected review specific criteria

Study	Ramsey, 2003[76]	Mvundura, 2010[77]	Ladabaum, 2011[78]	Wang, 2012[79]	Snowsill, 2014, 2015[4, 75]	Barzi, 2015[80]	Gallego, 2015[81]	Severin, 2015[82]
Diagnosis								
Patients are tested for *MLH1* mutations	✓	✓	✓	✓	✓	✓	✓	✓
Patients are tested for *MSH2* mutations	✓	✓	✓	✓	✓	✓	✓	✓
Patients are tested for *MSH6* mutations	✗	✓	✓	✓	✓	✓	✓	✓
Patients are tested for *PMS2* mutations	✗	✓	✓	✓	✓	✓	✓	✓
Appropriate informed consent and counselling is included	✓	✓	✓	✓	✓	?	✓	✓
The study considers patients declining counselling	?	✓	?	?	✓	?	✓	?
The study considers patients declining genetic testing	✓	✓	✓	✓	✓	✓	✓	✓
The effect of diagnostic errors is considered	✓	✓	✓	✓	✓	✓	✓	✓
The study considers the impact of a national strategy on the proportion of patients who do not already know their Lynch syndrome status	✗	✗	✗	✗	✓	✓	✗	✗
Management								
The study considers colorectal cancer	✓	✓	✓	✓	✓	✓	✓	✓
The study considers endometrial cancer	✗	✗	✓	✓	✓	✓	✗	✗
The study considers ovarian cancer	✗	✗	✓	✓	✗	✓	✗	✗
The study considers other Lynch-associated cancers	✗	✗	✗	✗	✗	✓	✗	✗
The study considers interactions of cancers appropriately	N/A	N/A	?	?	✗	?	N/A	N/A

Study	Ramsey, 2003[76]	Mvundura, 2010[77]	Ladabaum, 2011[78]	Wang, 2012[79]	Snowsill, 2014, 2015[4, 75]	Barzi, 2015[80]	Gallego, 2015[81]	Severin, 2015[82]
Colonoscopic surveillance in the study is explicitly justified (e.g., by reference to guidelines or clinical practice)	✓	✓	✗	✗	✓	✓	✗	✓
The study considers patients declining recommended surveillance	✓	✓	✓	✓	✓	✓	?	✓
The study considers the difference in incidence of CRC between males and females	✗	✗	✓	✓	✓	✓	✗	✓
The study considers the difference in incidence of Lynch-associated cancers between mutations of different MMR genes	✗	✗	✗	✗	✓	✗	✗	✗
The study accounts for the improved survival of Lynch syndrome CRCs relative to sporadic CRCs	?	✓	✓	✓	✓	✓	?	✓
The study considers the potential psychological impact of genetic testing	✗	✓	✗	✓	✓	✗	✗	✗

Key: N/A, not applicable

4.4 Discussion

The studies identified in this review report a wide variety of analyses, with varying quality in reporting. No single study answered our decision problem in full and the most common reason for this was that they did not including all the interventions identified by the NICE Scope or they were not from a UK perspective and therefore hard to generalise.

It is difficult to draw specific conclusions about which is the most cost-effective strategy, as this varies across studies and depends greatly on the willingness to pay threshold applied. Most studies stated that at least one strategy to identify Lynch syndrome could be cost-effective according to their perspective and when a universal genetic testing strategy was present, strategies that used tumour based tests to enrich the population appeared to improve cost-effectiveness (reducing ICERs). Most models agreed that effectiveness of colonoscopy screening, number of relatives and prevalence of Lynch syndrome impacted the cost-effectiveness of the models the most.

4.5 Conclusions

The economic analysis which came closest to answering the current decision problem was Snowsill et al. (2014). However, this requires updating to answer fully the current problem posed in the NICE Scope. Therefore our approach is to further adapt and develop the model created by Snowsill et al. to suit the current decision problem.

5 Independent economic assessment

5.1 Methods

The economic assessment reported in this chapter builds upon the model described in Snowsill et al. (2014, 2015).[4, 75] Where the model has not changed, reference is made to these documents, including direct quotes, and therefore these aspects are only briefly described here for clarity. Focus instead is given to alterations to the model.

5.1.1 Population

The base case population specified by the NICE Scope includes all newly-diagnosed colorectal cancer patients (probands) and their biological relatives (henceforth referred to as 'relatives'). This is a broader population than that previously specified in Snowsill et al. (2014), which considered only colorectal cancer (CRC) patients under 50 years old, and their relatives.

Four subgroups based on age (<50 years, <60 years, <70 years, ≥70 years) were also specified in the Scope and are presented in this report as subgroup analyses, separate from the main base case analysis. Parameters that have been identified as age-dependent are reported as such, and include: number of probands; proportions of probands and relatives who are men; and prevalence of Lynch syndrome in the population. Where data is unavailable for age subgroups, the base case values are used.

5.1.1.1 Number of probands

To estimate the number of probands, we used the same approach previously used in Snowsill et al. (2014), where the number of probands is taken from the most recent ONS Cancer Registration Statistics for England (2014).[21] The figures for the overall population and age subgroups are given in *Table 26*.

Table 26: Number of probands by age subgroup

Age subgroup	Number CRC registrations	Proportion men (Pooled ONS data 2006-2014)
Base case (no age limit)	34,025	55%
Under 50 years	2,107	52%
Under 60 years	5,880	55%
Under 70 years	13,823	58%
Over 70 years	20,202	53%

5.1.1.2 Number of relatives

The method for estimating the number of relatives was discussed in detail in Snowsill et al. (2014). In summary, the number of relatives included in the model represents the possible group of identifiable relatives. For example, for probands in whom a Lynch syndrome mutation is identified, this is the possible number of relatives who are both contactable and may be identified through cascade testing.

The number of relatives in Snowsill et al. (2014) was set to 5, based on previous data from Barrow et al. (2009) (2.35 relatives per proband) and unpublished data provided by Ian Frayling. Data recently collected from the Manchester regional Lynch syndrome registry, as part of a PhD thesis investigating the diagnosis of Lynch syndrome, suggested an average of 9.95 relatives per index patient.[84] As such the base case number of relatives is increased slightly to 6 to better account for this new information. As in Snowsill et al., this number is varied from 0 to 12 in univariate sensitivity analysis, to acknowledge the wide variation in the data sources. The number of relatives per proband is assumed not to alter with the age of the proband, given a paucity of evidence to demonstrate otherwise.

The Manchester Lynch syndrome registry estimated an average of eight FDRs per index patient,[84] but this seems exceptionally high, so we assume this figure actually represents the result of cascade testing to identify index patients. As such our estimated number of FDRs remains as 42% of all relatives (2.5 relatives per proband), which is based on a combination of published (Jenkins et al., 2006; Hampel et al., 2008)[85, 86] and unpublished (supplied by Ian Frayling, Cardiff University, 2012) data, as reported in Snowsill et al. (2014).

The proportion of relatives who are male was taken from pooled analysis of unpublished data (supplied by Ian Frayling, Cardiff University, 2012; Munaza Ahmed, Wessex Clinical Genetics Service, University Hospital Southampton NHS Trust, 2012) giving a value of 38%, as previously used in Snowsill et al. (2014).

5.1.1.3 Prevalence of Lynch syndrome

The prevalence of Lynch syndrome among CRC probands is estimated from Hampel et al. (2008),[85] as in Snowsill et al. (2014). The base case value (2.8%) and the age subgroup values are presented in *Table 27*.

In Snowsill et al. (2014) it was important to subdivide the proportion of the population with Lynch syndrome by mutation, as several parameters relied upon this information, including sensitivity and specificity of MSI and IHC. In the current analysis, the test accuracy is assumed to apply for all mutations, as new test accuracy evidence has not been available to predict the effect these mutations may have. This is a result of restricting the test accuracy studies to those that answer the decision problem, and not including studies with high-risk input populations. Mutation type does still affect some costs, and therefore is still included in the model.

As in Snowsill et al. (2014), the estimates of each Lynch syndrome mutation are taken from the supplementary evidence in the EGAPP review (2009), which stated that for all true Lynch syndrome-positive patients, 32% have a *MLH1* mutation, 39% a *MSH2* mutation, 14% a *MSH6* mutation and 15% a *PMS2* mutation.[39] Reported family registry data can differ significantly from these values (*Table 28*), particularly with respect to the proportion of *PMS2* mutations identified. This may potentially be due to *PMS2* testing having historically occurred less in current practice than in the trials on which the EGAPP review bases their values. Therefore, if systematic testing were more common in UK practice, this figure may change. We explore the possibility of the proportion of each mutation being closer to the reported registry data in a sensitivity analysis, using the Manchester Lynch syndrome registry data, as this is UK based data.[84]

Table 27: Prevalence of Lynch syndrome in CRC population

Age subgroup	Prevalence of LS in CRC population (%)
Base case (no age limit)	2.8
Under 50 years	8.4
Under 60 years	5.7
Under 70 years	3.8
Over 70 years	1.1

Source: Hampel et al. (2008)[85]

Table 28: Lynch syndrome-positive population by mutation

Source	*MLH1*	*MSH2*	*MSH6*	*PMS2*	Notes
EGAPP review supplementary evidence[39]	32%	39%	14%	15%	Based on trial data
Sjursen et al. (2010)[87]	18%	50%	26%	6%	Norway registry data (up to 2009)
Sjursen et al. (2016)[88]	36%	44%	17%	2%	New South Wales, Australia registry data (up to 2010)
Barrow (2015)[84]	40%	46%	11%	2%	Manchester, UK registry data (up to 2013)

For relatives of probands with Lynch syndrome, the proportion of relatives expected to test positive is estimated using a meta-analysis of studies (shown in *Table 29*), as reported in Snowsill et al. (2014) and gives a value of 44%. This value falls below 50% for a number of reasons, as reported in Snowsill et al. (2014), including:

> - de novo mutations can occur, which mean that no relatives of the index case will have the mutation [in the study by Jenkins and colleagues (2006),[86] 1 of 18 probands had a de novo mutation]
> - non-paternity can occur
> - mortality bias can occur, meaning that mutation carriers are more likely to have died before being able to receive predictive testing.
>
> *—Page 143 of Snowsill et al. 2014[4]*

Table 29: Meta-analysis of proportion of relatives testing positive

Study	Proportion (%)	95% CI (%)
Jenkins et al. 2006	44.1	37.0 to 51.5
Hampel et al. 2008	43.8	37.7 to 50.0
Ian Frayling (unpublished)	40.4	31.5 to 49.7
Munaza Ahmed (unpublished)	45.5	40.1 to 50.9
Random-effects meta-analysis	44	40.7 to 47.4

Source: Snowsill et al. (2014)

5.1.1.4 Age on entry

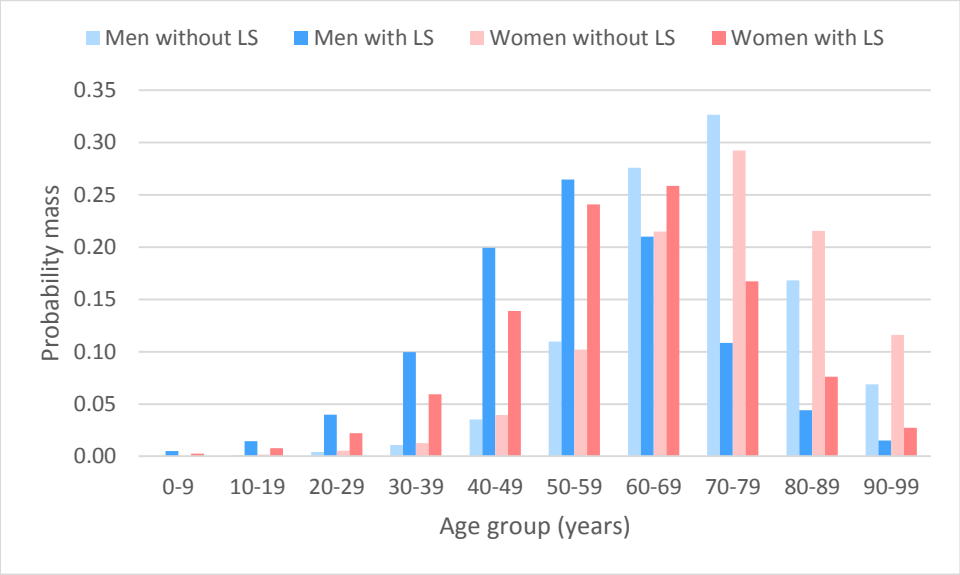
In the base case, there is no age threshold on colorectal cancer patients for screening for Lynch syndrome.

The age on entry for probands is based on the age distribution of CRC diagnoses. For probands without Lynch syndrome, the age distribution is estimated from cancer registration statistics in England from 2006 to 2014.[21, 89-96] These statistics are grouped into 5-year age groups. It was assumed that registrations were uniform within these age groups.

For probands with Lynch syndrome, the age distribution is estimated from the parametric colorectal cancer incidence function (see *Incidence rates for individuals with Lynch syndrome*, *page 170*).

The cumulative registrations were used to estimate an empirical cumulative incidence function. The cumulative incidence function was truncated as appropriate for the different age subgroups.

Figure 7: Age distributions of simulated probands



Key: LS, Lynch syndrome

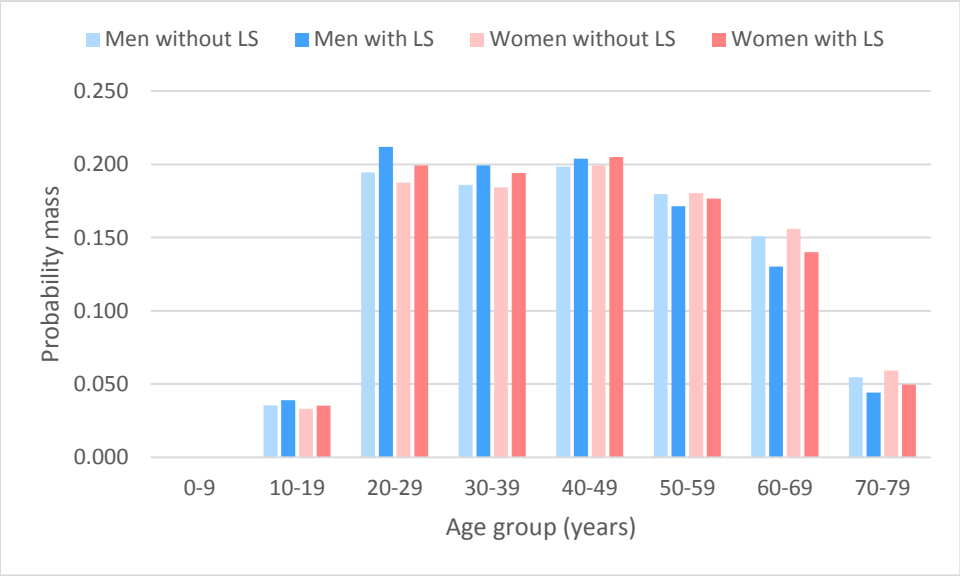
For relatives without Lynch syndrome, the age distribution was assumed to be equal to the age distribution of the general population, which was taken from the mid-2014 population

estimates for England[97] and mid-2014 population estimates of the very old (including centenarians) for the UK.[98]

For relatives with Lynch syndrome, the age distribution was estimated by multiplying the population estimate for the general population by an estimate of the CRC mortality-free survival for individuals with Lynch syndrome (i.e., incorporating the raised incidence of CRC in relatives with Lynch syndrome).

As previously,[4] the age distribution of relatives was right-truncated at 75 years as it is unlikely any intervention would be offered to individuals over 75 years, and was left-truncated at 18 years as few relatives are offered predictive testing before age 18.

Figure 8: Age distribution of simulated relatives



Key: LS, Lynch syndrome

5.1.2 Model structure

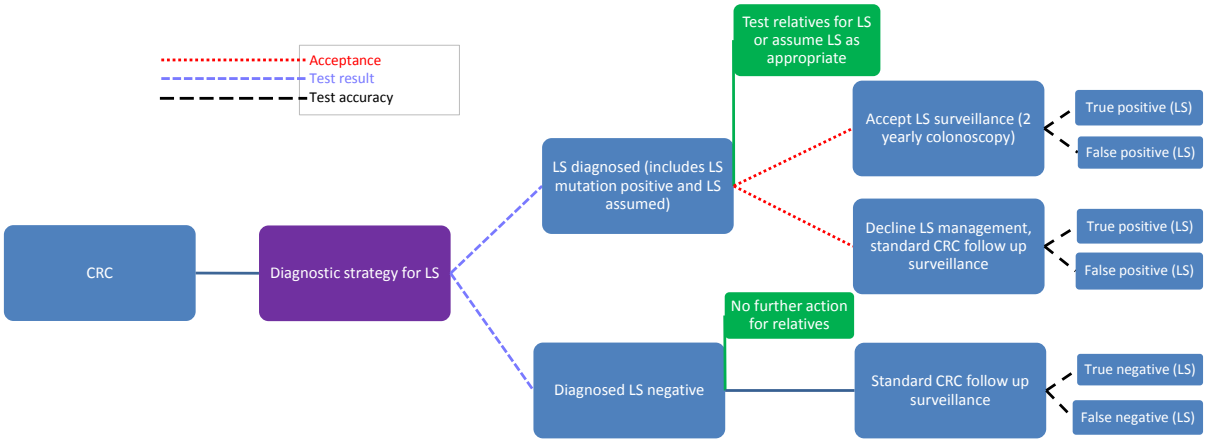
The model structure remains broadly similar to that reported in Snowsill et al. (2014, 2015).[4, 75] The model comprises two distinct sections: a decision tree model to investigate the short term outcomes of strategies to identify Lynch syndrome patients; and an individual patient simulation model to assess the long term implications of strategies to identify and manage Lynch syndrome. The model was built in Excel (Microsoft Corporation, Redmond, USA). A brief summary of the model structure and parameters is given in the following sections, with detailed discussion of any changes made to the model.

5.1.2.1 Diagnostic testing model

The section of the model that calculates diagnoses is built as a decision tree with no time component included. As in Snowsill et al. (2014), this assumes diagnosis of Lynch syndrome in probands and relatives occurs instantaneously, though in reality this may take up to several months or years. As before, it is also assumed that probands' treatment will not be influenced by a Lynch syndrome diagnosis, following the assumption that the results of Lynch syndrome testing will be unavailable prior to treatment. In most cases this treatment will be surgical resection with the possibility of chemotherapy or radiotherapy, depending on the stage of the cancer.

An overview of the diagnostic model is given in *Figure 9*. This is unchanged from Snowsill et al. (2014).[4]

Figure 9: Lynch syndrome diagnostic pathway



Key: CRC, colorectal cancer; LS, Lynch syndrome

5.1.2.1.1 Diagnostic strategies for probands

The NICE Scope for this project specifies diagnostic strategies for probands that are significantly altered from those reported in Snowsill et al. (2014).

The new strategies considered are:

1. No systematic testing to identify Lynch syndrome (all probands assumed to not have Lynch syndrome)
2. IHC four panel test for MLH1, MSH2, MSH6 and PMS2, followed by genetic testing if IHC result abnormal.
3. IHC four panel test, followed by *BRAF* testing for abnormal *MLH1* results. Genetic testing is done for any other (not *MLH1*) abnormal IHC result *or* for a negative *BRAF* test (negative for V600E).
4. IHC four panel test, followed by *MLH1* promoter hypermethylation testing for abnormal *MLH1* results. Genetic testing is done for any other (not *MLH1*) abnormal IHC result *or* for a negative *MLH1* promoter hypermethylation test.
5. IHC four panel test, followed by *BRAF* testing for abnormal *MLH1* results. A negative *BRAF* test (negative for V600E), is followed with *MLH1* promoter hypermethylation testing. Genetic testing is done for any other (not *MLH1*) abnormal IHC result *or* for a negative *MLH1* promoter hypermethylation test.
6. MSI test, followed by genetic testing for MSI result.
7. MSI test, followed by *BRAF* testing for MSI result. Genetic testing occurs for a negative *BRAF* test (negative for V600E).
8. MSI test, followed by *MLH1* promoter hypermethylation testing for MSI result. Genetic testing occurs for a negative *MLH1* promoter hypermethylation test.

9. MSI test, followed by *BRAF* testing for MSI results. A negative *BRAF* test (negative for V600E), is followed with *MLH1* promoter hypermethylation testing. Genetic testing is done for a negative *MLH1* promoter hypermethylation test.
10. Universal genetic testing (i.e., as first and only test for all probands)

These strategies are presented in *Figure 10* to *Figure 13*.

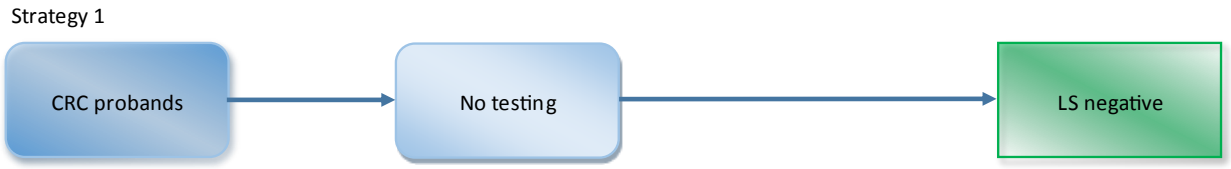
Strategies 5 and 9 include both *BRAF* and *MLH1* methylation testing. These are performed in sequence with either test able to rule out Lynch syndrome, e.g., a patient with *BRAF* V600E would not receive further testing. The ordering of these tests was informed by discussion at the NICE scoping workshop (11 January 2016).

In clinical practice, it is expected that a number of patients with Stage II CRC will undergo MSI or IHC analysis to inform treatment options. To highlight this, the model costs for MSI and IHC testing for all Stage II CRC patients (~27% of probands) in Strategy 1 (no testing), but assumes that this does not lead to any further testing for Lynch syndrome.

For the purposes of the base case, it is assumed that a MSI result corresponds to MSI-H (MSI-High). This assumption is explored in a scenario analysis where MSI results corresponds to MSI-L (MSI-Low), and this is detailed further in the model parameters section.

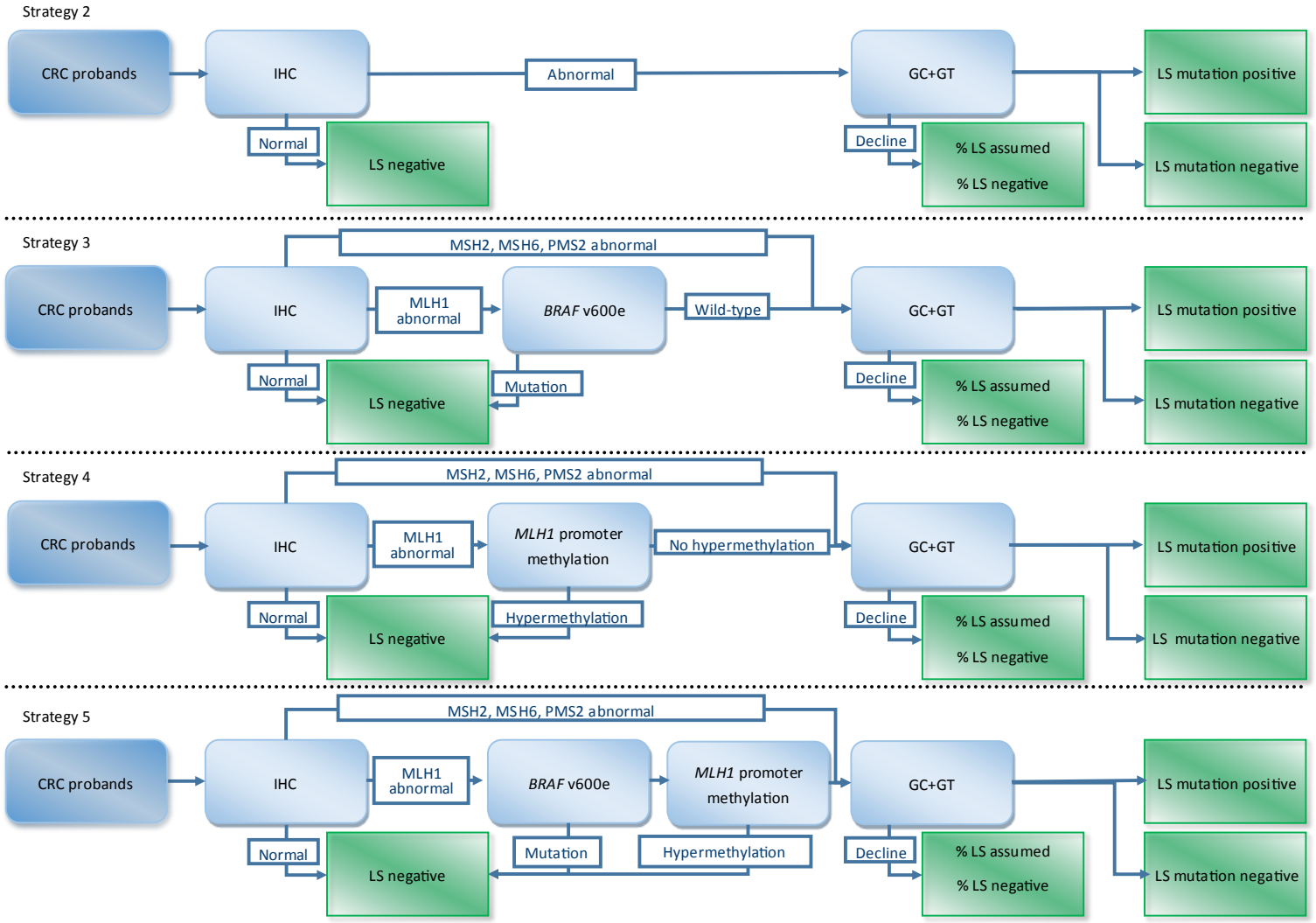
A certain proportion of probands in the MSI strategies are assumed to receive IHC to help interpret their genetic test results. For example, a variant of uncertain significance may be identified in *MSH2* and immunohistochemistry conducted to identify whether the tumour cells were MSH2-deficient (indicating the variant is likely to be pathogenic). Clinical opinion appears to be that this value can be quite low, so the value is set to 5% in the base case, on advice from clinical opinion (<5%, Ottie O'Brien, Northern Molecular Genetics Service; 10%, Samantha Butler, West Midlands Regional Genetics Laboratory). The impact of this parameter is explored in univariate sensitivity analysis, varying it from 0% to 10%.

Figure 10: Proband diagnostic strategy, no testing



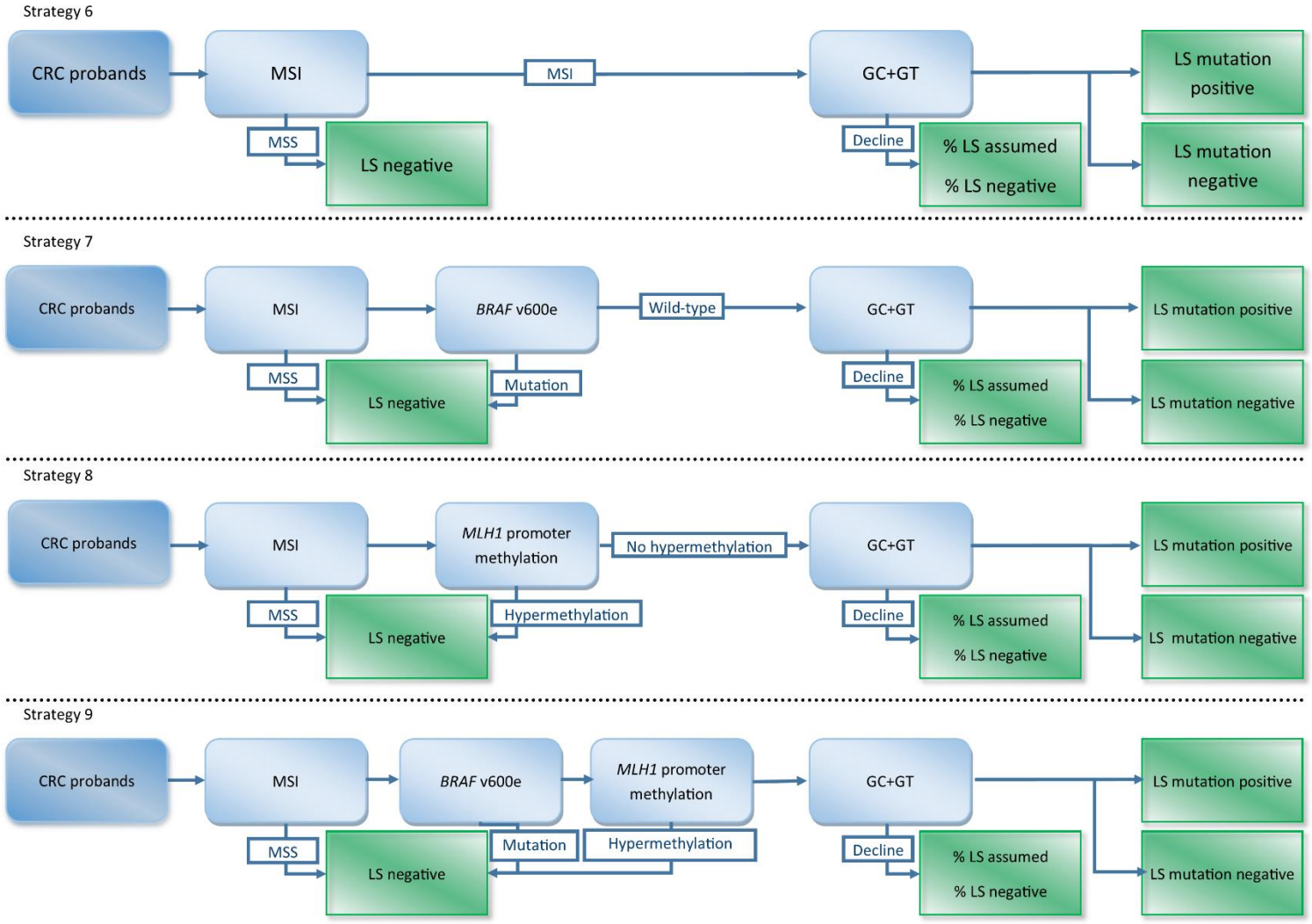
Key: CRC, colorectal cancer; LS, Lynch syndrome

Figure 11: IHC based diagnostic strategies for probands



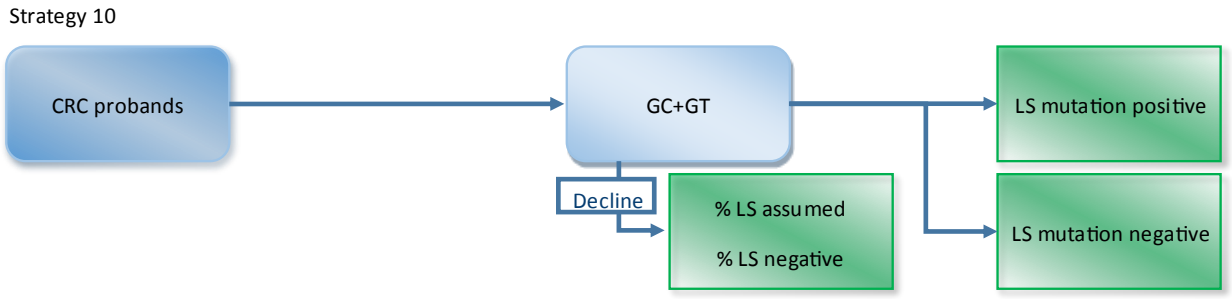
Key: CRC, colorectal cancer; GC, genetic counselling; GT, genetic testing; IHC, immunohistochemistry; LS, Lynch syndrome

Figure 12: MSI based diagnostic strategies for probands



Key: CRC, colorectal cancer; GC, genetic counselling; GT, genetic testing; LS, Lynch syndrome; MSI, microsatellite instability; MSI, microsatellite stable

Figure 13: Universal genetic testing strategy for probands



Key: CRC, colorectal cancer; GC, genetic counselling; GT, genetic testing; LS, Lynch syndrome

The potential results of the testing strategies for patients are the same as previously: LS-mutation positive, LS-assumed and LS-negative.[4] LS-mutation positive occurs when a proband receives a positive genetic test result and LS-negative occurs when a proband is ruled out by one of the tests in the strategy.

In Snowsill et al. (2014), 'LS-assumed' could occur either when genetic testing was uninformative or simply not done (the proband declines testing). A negative genetic test result was assumed to be uninformative for probands, meaning that although the test did not detect Lynch syndrome, it did not rule it out. To decide if the proband was LS assumed, the Amsterdam Criteria II (ACII) was used as an additional test, for those probands who were LS mutation negative, or who declined genetic testing. In this update to the model, any use of the Amsterdam Criteria II has been removed, in line with the focus of the decision problem to not look at using clinical criteria in the strategies. It is also now assumed that only probands who decline testing can become LS assumed, and this is set to 10% for all patients (lower than the average proportion of probands who were LS-assumed after declining testing in the 2014 model [21%]). We adjust this number for each age subgroup, using the three age limit scenarios from Snowsill et al. (21% for probands under age 50 years, 17% under 60 years, 13% under 70 years) and set the value for the over 70 subgroup to a significantly lower value (5%) to reflect that in higher age groups, few people are likely to be diagnosed with Lynch syndrome without genetic testing.

Unlike in Snowsill et al. (2014), where testing for *PMS2* was only modelled for probands who were negative for *MLH1*, *MSH2* and *MSH6*, but who had family history indicative of Lynch syndrome, it is assumed that all patients who accept genetic testing will receive testing for all four known genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*). The exception to this is that probands who follow strategies which use IHC followed by either *BRAF* V600E or *MLH1* promoter hypermethylation testing, will receive only *MLH1* and *PMS2* germline testing. Mutations related to *EPCAM* are assumed to be identified via the testing for *MSH2*. This is believed to be in line with current clinical practice, or where clinical practice differs from this, the model will overestimate costs associated with tumour-based testing strategies.

As in Snowsill et al. (2014), only a proportion of patients diagnosed as LS positive or LS-assumed accept an offer of LS surveillance.

5.1.2.1.2 Testing outcomes for probands

The primary outputs from the short-term model for each testing strategy that lead into the survival (i.e., long-term) section of the model remain the same as those reported in Snowsill et al. (2014):

- number of probands with LS receiving LS surveillance
- number of probands with LS not receiving LS surveillance (probands will receive some surveillance in line with BSG guidelines[43]; these are split into those identified as LS positive, but declined surveillance and those who were diagnosed LS negative
- number of probands without LS receiving LS surveillance
- number of probands without LS who do not receive LS surveillance (probands will receive some surveillance in line with BSG guidelines[43]); these are split into those identified as LS positive, but declined surveillance and those who were diagnosed LS negative.

Other outcomes include overall sensitivity and specificity of each diagnostic strategy.

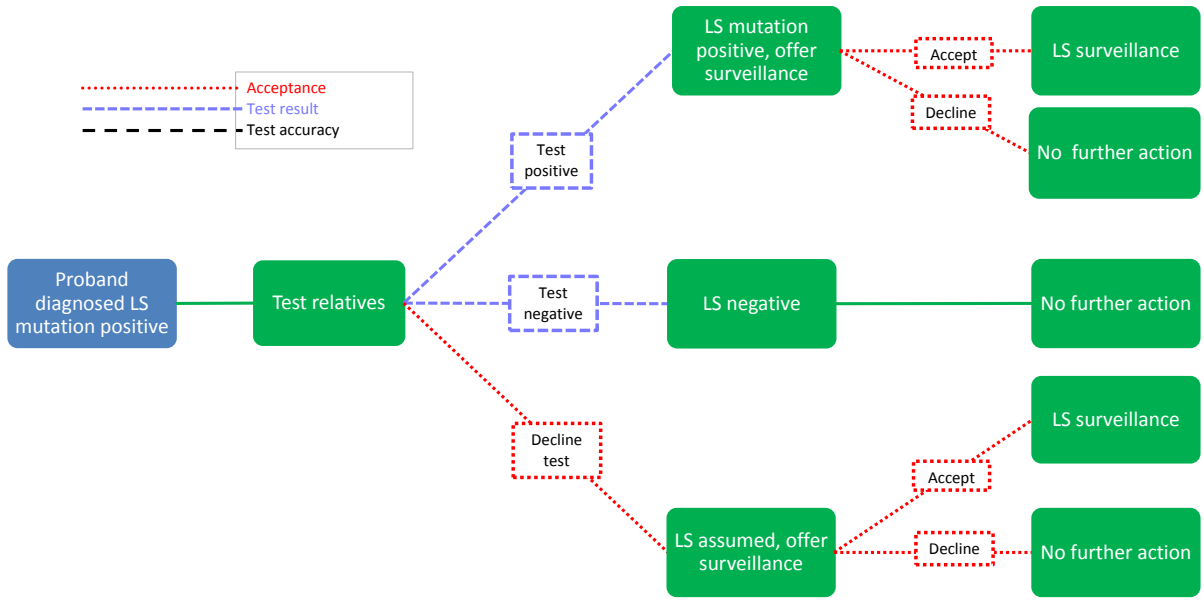
—Page 98 of Snowsill et al. 2014[4]

Probands who are diagnosed with Lynch syndrome (either mutation positive or assumed), but refuse surveillance may still be offered prophylactic surgery for metachronous CRC (mCRC) or endometrial cancer (EnCa).

5.1.2.1.3 Diagnostic strategies for relatives

These strategies are unchanged from Snowsill et al. (2014) and are summarised in *Figure 14* to *Figure 16*. As with probands, relatives can decline any testing or LS surveillance offered to them.

Figure 14: Diagnostic strategy for relatives of probands diagnosed as Lynch syndrome mutation positive



Key: LS, Lynch syndrome

Figure 15: Diagnostic strategy for relatives of probands diagnosed as Lynch syndrome assumed

Key: FDRs, first-degree relatives; LS, Lynch syndrome

Figure 16: Diagnostic strategy for relatives of probands diagnosed as Lynch syndrome mutation negative

Key: LS, Lynch syndrome

5.1.2.1.4 Testing outcomes for relatives

As stated in Snowsill et al. (2014):

> The primary short term model outputs are:
>
> - number of relatives with LS receiving LS surveillance
> - number of relatives with LS not receiving LS surveillance (split into those identified as LS positive, but declined surveillance and those who were diagnosed LS negative)
> - number of relatives without LS receiving LS surveillance
> - number of relatives without LS who do not receive surveillance (split into those identified as LS positive, but declined surveillance and those who were diagnosed LS negative)
>
> The sensitivity and specificity for each testing strategy for relatives is recorded.
>
> *—Page 100 of Snowsill et al. 2014*[4]

5.1.2.2 Long-term outcomes model

As previously described,[4] long-term outcomes are modelled for all probands and relatives regardless of the diagnostic path they follow. An individual patient sampling model is used to simulate 240,000 patients, distributed across 24 groups, representing all combinations of the following variables as shown in *Table 30*. An individual patient sampling model is justified because no patient interactions are modelled, but there are too many patient states for a cohort model.

Table 30: Patient groups in the long-term outcomes model

Variable	Values
Patient type	• Proband • Relative
Actually has Lynch syndrome	• Yes • No
Lynch syndrome diagnosis and management	• Diagnosed and surveillance colonoscopies accepted • Diagnosed and surveillance colonoscopies not accepted • Not diagnosed
Sex	• Male • Female

Mean long-term outcomes are then estimated for each of the 24 groups. These are then used to estimate the long-term outcomes for each of the diagnostic strategies in the decision tree.

Patients are simulated for one year at a time. Each patient starts each year in a particular state which determines the events which can occur during that year. The events in turn determine costs incurred and the state of the patient for the next year if the patient is still alive. The hazard rate for events (except elective events such as surveillance colonoscopies) was assumed to be constant during each year, but could change between years.

Life years and QALYs are calculated based on the patient's state at the beginning of the year and any events occurring during the year, e.g., if a patient starts the year without cancer and develops cancer after 3 months, then the patient will accrue 3 months of life at non-cancer utility and 9 months with the utility decrement from cancer (assuming the patient does not die within the year).

Table 31 shows the different events included in the model. Mortality events are competing – no other events can occur after the patient dies.

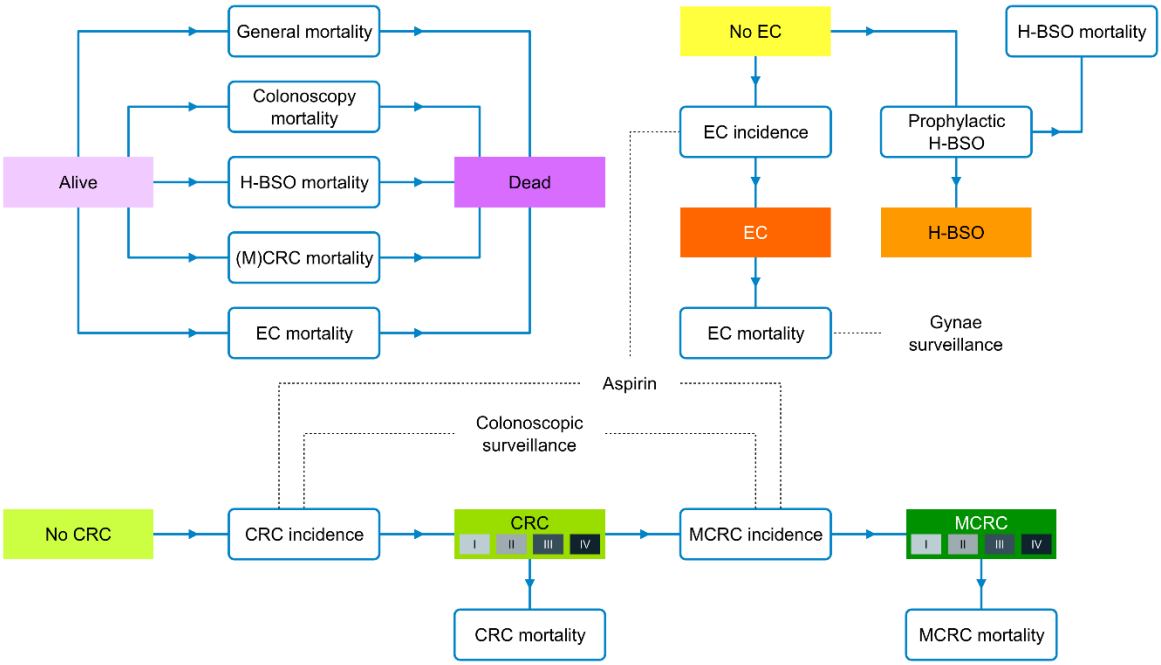
Table 31: Competing and non-competing events in the PenTAG model for different patient groups

Patient group	Competing events	Non-competing events
All patients	• General mortality	
Patients undergoing LS surveillance (aged 25–75)	• Mortality following colonoscopy	• Colonoscopy • Adverse events (includes bleeding and perforation) following colonoscopy
Patients with CRC (aged under 75)	• Mortality following colonoscopy	• Colonoscopy • Adverse events (includes bleeding and perforation) following colonoscopy
Patients with CRC	• CRC mortality	
Patients with an index CRC (without metachronous CRC)		• Metachronous CRC incidence
Patients without CRC		• CRC incidence
Women with Lynch syndrome without EC		• EC incidence
Women with Lynch syndrome with EC	• EC mortality	
Women diagnosed with Lynch syndrome without EC and without H-BSO	• Mortality following prophylactic H-BSO	• Commence gynaecological surveillance • Stop gynaecological surveillance • Decline risk reduction for gynaecological cancer • Prophylactic H-BSO

Key: CRC, colorectal cancer; EC, endometrial cancer; H-BSO, hysterectomy and bilateral salpingo-oophorectomy; LS, Lynch syndrome

Figure 17 presents a model diagram for the long-term outcomes model, indicating the mortality events and cancer incidence events, as well as prophylactic H-BSO. Gynaecological surveillance is indicated to reduce the risk of endometrial cancer mortality, but this is only if the individual is receiving surveillance prior to cancer incidence.

Figure 17: Simplified model diagram for the long-term outcomes model



Key: CRC, colorectal cancer; EC, endometrial cancer; gynae, gynaecological; H-BSO, hysterectomy and bilateral salpingo-oophorectomy; MCRC, metachronous colorectal cancer

5.1.2.2.1 Patient state

The state of each simulated individual at any time is defined by a number of properties, which collectively provide all the information necessary to select appropriate treatment pathways and calculate risks of events. The patient state is composed of:

- whether the patient is alive,
- patient's age (at the start of the year),
- patient's sex,
- patient's bowel state (defined below),
- patient's gynaecological state (defined below),
- patient's Lynch syndrome status and diagnosis status,
- patient's acceptance of Lynch syndrome surveillance colonoscopies if offered.

Patient's bowel state

The patient bowel state encapsulates whether the patient has a clinically diagnosed colorectal cancer and the extent of any bowel surgery. Though it is possible within an individual sampling model to track a number of primary colorectal cancers and their properties, we make the simplifying assumption that each patient will have no more than two primary colorectal cancers throughout their life (as in other decision models, e.g., Mvundura et al.[77]).

Each colorectal cancer is staged using the modified Dukes' stage (A–D) or American Joint Committee on Cancer (AJCC) stage (I–IV), which are effectively equivalent. The Tumour–

Node–Metastasis (TNM) system is used clinically to give finer staging detail, but incidence and survival statistics are currently not widely available for TNM stages.

The model tracks the stage of each colorectal cancer and the time since diagnosis (to determine the hazard of colorectal cancer mortality).

We model two portions of the bowel: the colon and the rectum. CRCs can develop in any portion of the bowel still intact. We model four surgery types, based on the extent of bowel removed (see *Table 32*). This is a small extension to the three surgery types used in Maeda et al.[99] to account for the fact that rectal cancer can be the first primary cancer in our cohort. *Section 5.1.2.2.7* (*page 176*) gives details on surgical management.

Table 32: Extent of bowel removed for included surgeries

Surgery	Bowel removed
Segmental colon resection	Part (but not all) of the colon
Subtotal colectomy	All of the colon
Anterior resection	All of the rectum
Proctocolectomy	All of the colon and rectum

Bowel state on entry

All probands enter the model with an index CRC (i.e., without a metachronous CRC). The Dukes' stage for probands is sampled randomly using the distribution described in *Stage on diagnosis* (*page 171*).

Probands entering the simulation are randomly assigned a surgical state in accordance with the estimated probability that they had colon cancer versus rectal cancer (*Table 33*) and the probabilities of different types of surgery for those cancers (*Table 34*). *Table 35* gives the resulting distribution of initial surgical states for probands entering the model, according to their sex and Lynch syndrome status.

Table 33: Probability that index CRC of proband entering PenTAG model is colon cancer (ICD-10 code C18)

Proband type	Male	Female	Source
With Lynch syndrome	0.94	0.94	Dinh online appendix[100]
Without Lynch syndrome			ONS Cancer registration statistics, England 2013[96]
Base case	0.63	0.72	
<50 years	0.61	0.70	
<60 years	0.56	0.66	
<70 years	0.57	0.68	
≥70 years	0.67	0.75	

Table 34: Surgery for CRC according to location in general population

Location of CRC	Surgery (% of cases)	Source
Colon	Segmental resection (96%) Subtotal colectomy (4%)	NHS Bowel Cancer Audit 2011[101]
Rectum	Anterior resection (98%) Proctocolectomy (2%)	NHS Bowel Cancer Audit 2011[101]

Table 35: Initial surgical state for probands entering the model

Surgery	With Lynch syndrome		Without Lynch syndrome	
	Men	Women	Men	Women
Segmental resection	0.907	0.907	0.603	0.696
Subtotal colectomy	0.033	0.033	0.022	0.026
Anterior resection	0.059	0.059	0.368	0.274
Proctocolectomy	0.001	0.001	0.007	0.005

Relatives enter the model without colorectal cancer, i.e., they are at risk of up to two colorectal cancers (index and metachronous). In reality some relatives would be survivors of previous colorectal cancer.

Table 36 gives an estimate of what proportion of relatives would be survivors of previous colorectal cancer. Estimates for relatives without Lynch syndrome are based on ten-year colorectal cancer prevalence published by the (UK) National Cancer Intelligence Network,[102] assuming that the proportion of CRC survivors with colon cancer is the same as the proportion of incident CRCs which are colon cancer. The prevalence of previous CRC for relatives with Lynch syndrome is estimated by multiplying by a scale factor of 38% / 2.6% = 14.8 for males and 31% / 1.7% = 18.1 for females, where 38% and 31% are estimates of the cumulative risk of CRC to age 70 for males and females with Lynch syndrome respectively[16] and 2.6% and 1.7% are estimates of the cumulative risk of CRC to age 70 for males and females without Lynch syndrome respectively, calculated using population, CRC incidence and CRC mortality statistics for England and Wales in 2010.[93, 103-105] Again it was assumed that the proportion of survivors with colon cancer would match the proportion of incident cases, this time estimated by Dinh et al.[100]

Relatives with previous CRC would experience a higher mortality rate and therefore preventing a further colorectal cancer would be expected to give a smaller life year gain than in relatives without previous colorectal cancer. These colorectal cancer survivors would be likely to have early stage CRC, to have undergone segmental resection and to be followed up for recurrence or metachronous cancer.

The model incorporates initial surgical states for relatives entering the model (see *Table 37*; proportions based on surgical choice for people not known to have Lynch syndrome and prevalence of colon and rectal cancer as in *Table 36*). Most relatives have no previous surgery (as most relatives have no previous CRC), and of those with previous surgery, the majority have a previous segmental resection which imparts no risk reduction in the model. A very small number ($\ll 1\%$) of relatives enter the model with previous surgery which does impart a risk reduction. As all these relatives enter with risk reduction irrespective of the diagnostic strategy this would decrease the potential life year gain of correctly identifying relatives as having Lynch syndrome; we therefore expect that including initial surgical states

has a very small (probably negligible) negative impact on cost-effectiveness of strategies identifying Lynch syndrome (i.e., ICERs for testing strategies slightly increased versus no testing).

Table 36: Estimated proportion of relatives who would have previously had colorectal cancer

CRC prevalence	With Lynch syndrome		Without Lynch syndrome	
	Men	Women	Men	Women
None	0.9683	0.9748	0.9979	0.9986
Colon cancer	0.0298	0.0237	0.0013	0.0010
Rectal cancer	0.0019	0.0015	0.0009	0.0004

Table 37: Initial surgical state for relatives entering the model

Surgery	With Lynch syndrome		Without Lynch syndrome	
	Men	Women	Men	Women
None	0.9683	0.9748	0.9979	0.9986
Segmental resection	0.0288	0.0229	0.0012	0.0009
Subtotal colectomy	0.0011	0.0008	0.0000	0.0000
Anterior resection	0.0019	0.0015	0.0009	0.0004
Proctocolectomy	0.0000	0.0000	0.0000	0.0000

Patient's gynaecological state (women only)

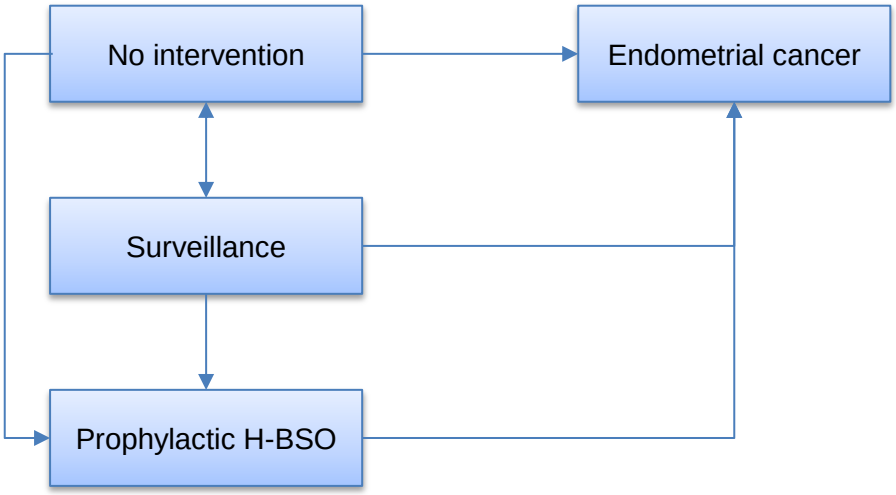
A patient's gynaecological state encapsulates what risk reducing measures they have employed, and whether they have had endometrial cancer.

Although endometrial cancer can be staged and there are some survival estimates according to stage,[106] the staging system has been changed recently and most patients are diagnosed in early stages. Therefore, the stage of endometrial cancer is not tracked in the model, but the time since diagnosis is tracked.

It is assumed that a patient will not get more than one primary endometrial cancer, since surgery will be hysterectomy, which is very effective at preventing endometrial cancer when used prophylactically.[25]

Risk reducing measures available to patients are gynaecological surveillance and prophylactic hysterectomy (and bilateral salpingo-oophorectomy). Patients can start and stop surveillance, but prophylactic hysterectomy is irreversible and surveillance cannot be performed in patients after hysterectomy (*Figure 18*).

Figure 18: Gynaecological state model diagram



Key: H-BSO, hysterectomy and bilateral salpingo-oophorectomy

Gynaecological state on entry

All simulated individuals are assumed to start without endometrial cancer.

Probands and relatives who have been diagnosed with Lynch syndrome may be offered surveillance or prophylactic hysterectomy and bilateral salpingo-oophorectomy depending on the age.

Table 38 shows the assumed distribution of risk reducing strategies according to age at diagnosis, which is estimated based on audit data from the Northern Genetic Service (Lorraine Cowley, Principal Genetic Counsellor, Newcastle upon Tyne Hospitals NHS Foundation Trust; 20th November 2012).

Table 38: Gynaecological cancer risk reduction for women with Lynch syndrome on entry

Age at Lynch syndrome diagnosis	No risk reduction	Surveillance	Prophylactic H-BSO
0–34	1.000	0.000	0.000
35–44	0.200	0.600	0.200
45–59	0.167	0.458	0.375
60–69	0.000	0.143	0.857
70+	0.143	0.000	0.857

Key: H-BSO, hysterectomy and bilateral salpingo-oophorectomy

5.1.2.2.2 Outcomes

For each of the 24 patient groups (described in *Section 5.1.2.2*, *page 159*), the following outcomes are recorded from the simulation:

- Costs (discounted and undiscounted),
- QALYs (discounted and undiscounted),
- Overall survival (and whether censored upon reaching age 100),

- Colorectal cancer-, endometrial cancer- and overall cancer-free survival (and whether censored due to death or reaching age 100),
- Event-free survival (and whether censored upon reaching age 100),
- Number of incident colorectal cancers,
- Number of incident endometrial cancers,
- Number of colonoscopies performed,
- Disaggregated costs (discounted and undiscounted).

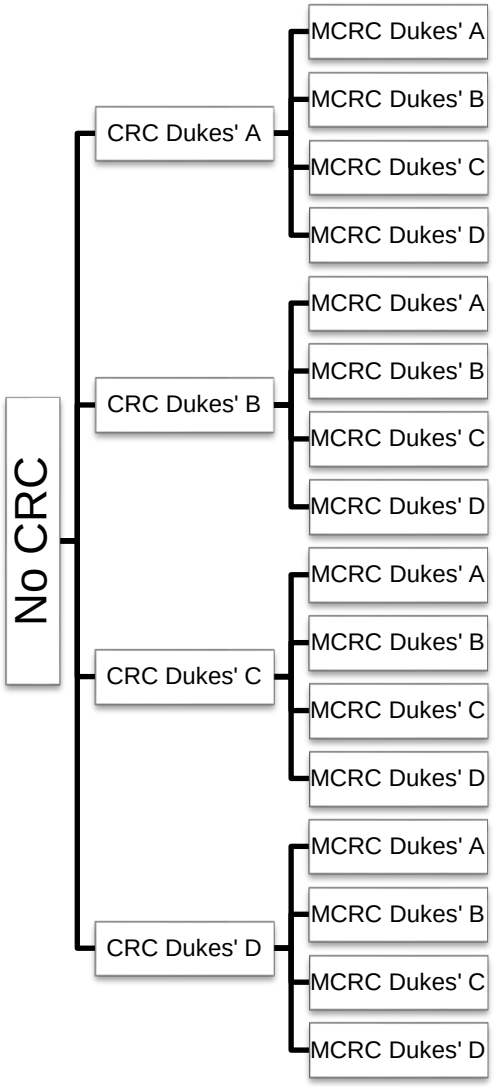
5.1.2.2.3 Colorectal cancer

The simulation model includes four events relating to colorectal cancer:

- Index colorectal cancer incidence;
- Index colorectal cancer mortality;
- Metachronous colorectal cancer incidence;
- Metachronous colorectal cancer mortality.

The incidence events transform the patient's bowel state (e.g., index colorectal cancer incidence transforms the bowel state from "No CRC" to "Index CRC" with a particular stage). The mortality events result in the patient dying with no further events occurring.

Figure 19: Colorectal cancer and metachronous colorectal cancer incidence model diagram



Key: CRC, colorectal cancer; MCRC, metachronous colorectal cancer

The probability that a colorectal cancer incidence event occurs within a year is dependent on the incidence rate, while the probability that a colorectal cancer mortality event occurs is dependent on the survival function.

Colorectal cancer incidence

Colorectal cancer incidence rates in the model are dependent on the following patient characteristics:

- Age;
- Sex;
- Whether the patients has had a previous CRC;
- Time since first CRC;
- Lynch syndrome status;

- Risk-reducing measures, i.e., regular colonoscopies and aspirin, as described in *Sections 5.1.2.2.7* (*page 176*) and *5.1.2.2.8* (*page 179*).

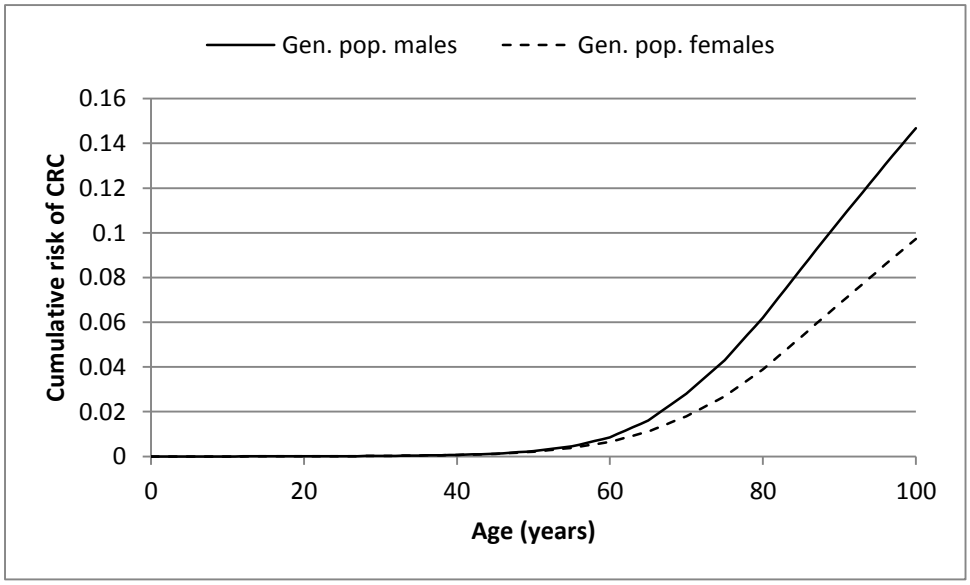
Different annual incidence rates are provided for the eight combinations of sex, previous cancer (yes/no) and Lynch syndrome status and then risk-reducing measures are incorporated as hazard ratios which have a simple multiplicative effect on the incidence rate.

Incidence rates for individuals without Lynch syndrome

The incidence rates for males and females without previous cancer without Lynch syndrome were estimated from pooled registration statistics for colorectal cancer in England between 2006 and 2014 inclusive[89-96] and the estimated population in the midpoints of those years.[107] Following the methodology adopted by the Office for National Statistics[93] we calculate the age-specific rate of colorectal cancer incidence by dividing the number of colorectal cancer registrations within a time period by an estimate of the person-years lived during that period. Incidence figures were pooled across five years to achieve a large sample size but not further back than 2006 as such data may not reflect more recent developments in cancer detection and registration.

Cancer registration statistics are not provided for each year of age but for age groups, generally of five years. We assumed that within each of these age groups the incidence rate would remain constant. The resulting cumulative risk of CRC for individuals without Lynch syndrome is shown in *Figure 20*.

Figure 20: Cumulative risk of colorectal cancer for individuals without Lynch syndrome



Note: Does not account for non-CRC mortality
Key: CRC, colorectal cancer; gen. pop., general population

We estimated the incidence of metachronous colorectal cancer (i.e., incidence in individuals who had a previous colorectal cancer) for individuals without Lynch syndrome by adjusting the incidence of first CRC by a hazard ratio of 1.4 for the first three years after first CRC and 1.3 for the following seven years, from Mulder et al.[108] Mulder et al. studied 10,283 Dutch patients with CRC undergoing standard follow-up. After 10 years no additional hazard was applied.

Incidence rates for individuals with Lynch syndrome

Previously,[4] we conducted a literature review to identify studies from which the age-dependent incidence rates for individuals with Lynch syndrome could be estimated.

Subsequent reviews[2, 17, 109] have not identified any additional studies.

Møller et al.[5] have subsequently published estimates of the cancer risk for individuals with Lynch syndrome while undergoing colonoscopic surveillance – this does not address the need here (i.e., for the cancer risk in the absence of interventions).

In the absence of new evidence to consider, the model includes an incidence rate for colorectal cancer based on Bonadona et al. (2011).[16]

A logistic model for cumulative risk was fitted to data from Bonadona et al., using the following parameterisation and ordinary least-squares regression:

$$F(x) = \frac{\beta_0}{1 + \exp\left(-\beta_1(x - \beta_2)\right)}$$

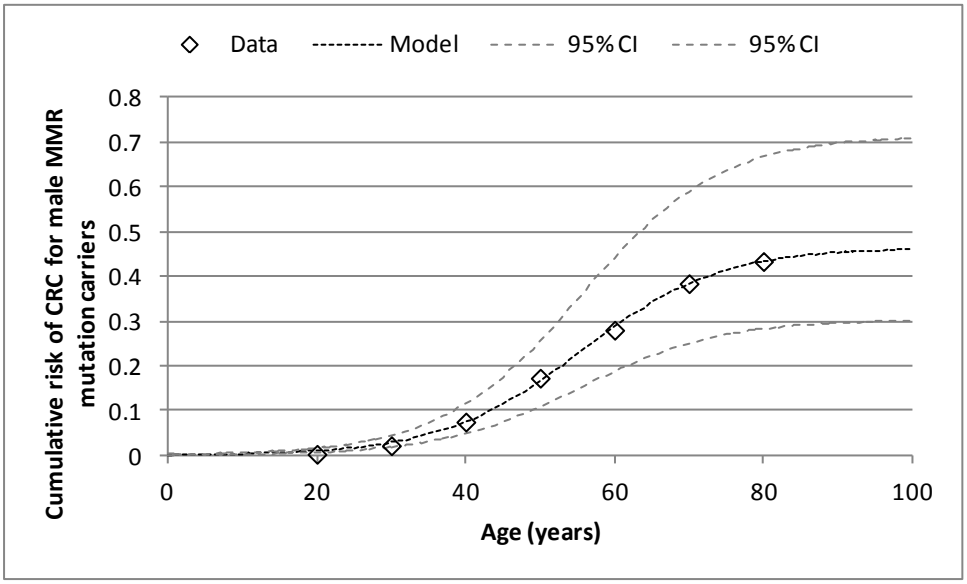
The 95% confidence intervals for the cumulative risk to age 70 from Bonadona et al. were used in sensitivity analyses (by varying β_0 appropriately). *Table 39* shows the parameters used in the base case and sensitivity analyses and *Figure 21* and *Figure 22* graphically show the fit to the data from Bonadona et al. (2011).

Table 39: Logistic model parameters for colorectal cancer incidence in individuals with Lynch syndrome

Parameter	Base case	Sensitivity analyses
β_0	M 0.464 F 0.435	M 0.303, 0.715 F 0.265, 0.697
β_1	M 0.107 F 0.108	
β_2	M 55.5 F 61.3	

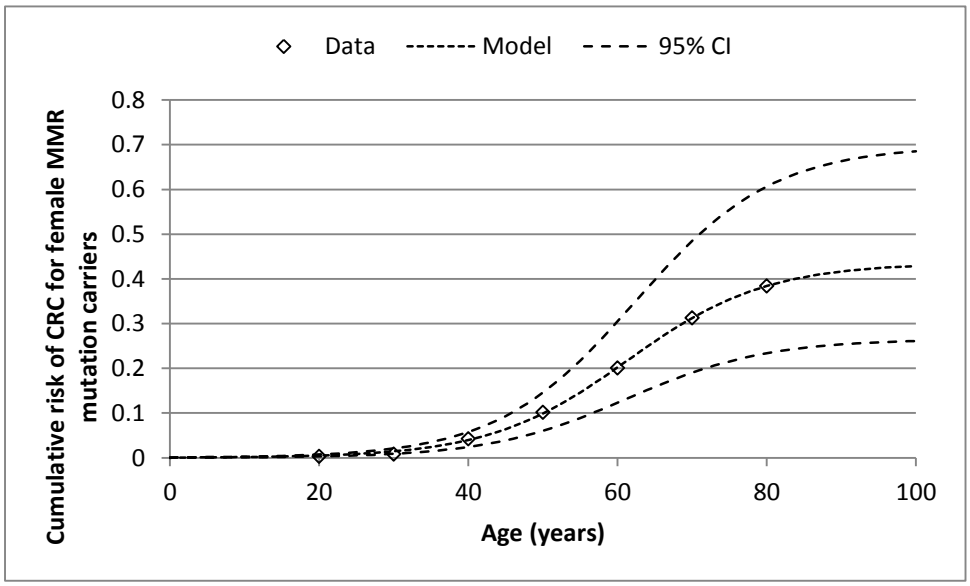
Key: F, women with Lynch syndrome; M, men with Lynch syndrome

Figure 21: Colorectal cancer incidence for men with Lynch syndrome



Key: CI, confidence interval; CRC, colorectal cancer; MMR, mismatch repair

Figure 22: Colorectal cancer incidence for women with Lynch syndrome



Key: CI, confidence interval; CRC, colorectal cancer; MMR, mismatch repair

Stage on diagnosis

The colorectal cancer stage on diagnosis is an important predictor of survival. Historically the modified Dukes' stage (A–D) has been used for cancer staging, but it is more common now to refer to stages by Roman numerals (I–IV).

We assumed that the stage on diagnosis would be independent of the age, sex, Lynch syndrome status and whether it was the first (index) CRC or a metachronous CRC (Fajobi et al.[110] conclude stages for metachronous CRC are no worse than for index CRC and there was no consensus on whether they might be better). We also assumed stage on diagnosis of metachronous CRC was independent of the stage of the index CRC and that the stage

was independent of the colorectal cancer site. CRC stage was assumed to depend only on whether the person was undergoing Lynch syndrome surveillance colonoscopies.

The stage distributions are given in *Stage on diagnosis* (*page 186*).

Colorectal cancer site

As stated in *Patient's bowel state* (*page 162*) we model two sections of the bowel, the colon and the rectum. We grouped rectosigmoid cancer (ICD-10 code C19) into rectal cancer. The site of incident CRCs was dependent on sex, whether the person has Lynch syndrome and any previous surgery (see *Table 40*).

Table 40: Probability incident CRC is situated in the colon

Previous surgery	With Lynch syndrome	Without Lynch syndrome	
		Men	Women
None	0.94	0.63	0.72
Segmental resection	0.94	0.63	0.72
Subtotal colectomy	0.00	0.00	0.00
Anterior resection	1.00	1.00	1.00
Proctocolectomy	N/A	N/A	N/A

Notes: N/A as zero CRC incidence following proctocolectomy

It is assumed that all CRCs are colon cancers following anterior resection and that all CRCs are rectal cancers following subtotal colectomy. If there is no previous surgery or a previous segmental resection the probability of the CRC being situated in the colon for a person with Lynch syndrome is estimated as 0.94 based on Dinh et al.[100] For males and females without Lynch syndrome the probability of colon cancer is estimated from ONS cancer registration statistics.[93]

Colorectal cancer survival

We assume that mortality due to colorectal cancer depends on the following:

- CRC stage at diagnosis,
- Years since diagnosis,
- Age at diagnosis, and
- Lynch syndrome status (see *Lynch syndrome colorectal cancer survival*, *page 174*).

We did not consider the effect of the following on mortality due to colorectal cancer:

- Patient's sex,
- Site of CRC, and
- Surgery for CRC.

The baseline annual rate of mortality due to CRC was derived from data provided by the (UK) National Cancer Intelligence Network[102] by extracting 1-, 2-, 3-, 4- and 5-year relative survival from survival curves and assuming constant rates of mortality within each year (*Table 41* and *Figure 23*). It was assumed that the mortality rate for 4-5 years since diagnosis also applies after 5 years (*Table 42*).

Table 41: Relative survival of patients with colorectal cancer by Dukes' stage across all ages

Years since diagnosis	Dukes' A	Dukes' B	Dukes' C	Dukes' D
1	0.969	0.917	0.815	0.380
2	0.965	0.872	0.681	0.193
3	0.957	0.831	0.583	0.116
4	0.945	0.799	0.522	0.083
5	0.932	0.770	0.477	0.066

Source: National Cancer Intelligence Network[102]

Figure 23: CRC survival in the model

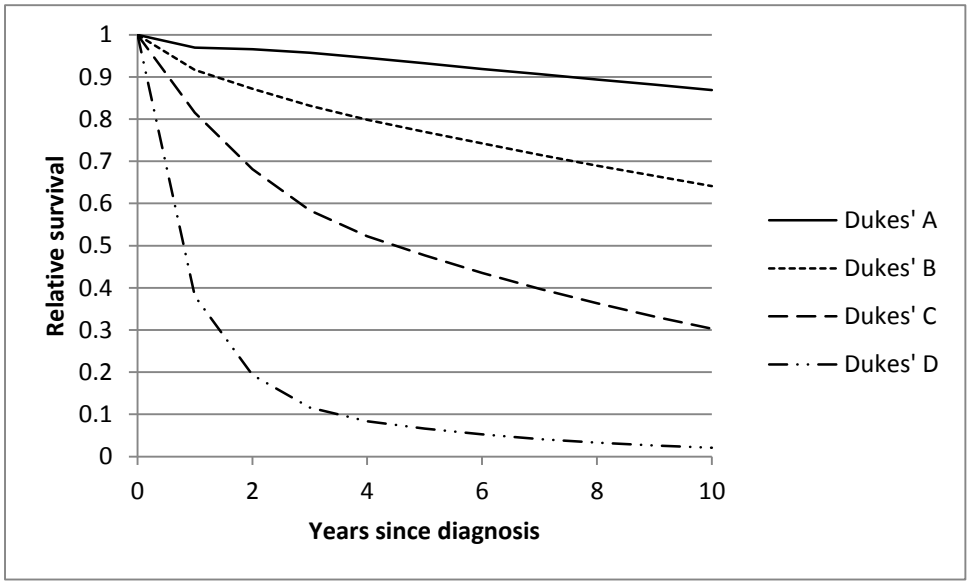


Table 42: Mortality rate from CRC (per 100,000 person years) by Dukes' stage

Years since diagnosis	Dukes' A	Dukes' B	Dukes' C	Dukes' D
0-1	3,102	8,709	20,460	96,729
1-2	419	5,000	17,971	67,733
2-3	843	4,761	15,465	51,116
3-4	1,279	4,000	11,060	32,857
Over 4	1,400	3,667	9,068	23,375

The assumption that the mortality rate after 5 years is equal to the mortality rate for 4-5 years is likely to be a slight overestimate of CRC mortality (see *Table 43*). The result of a slight overestimate of CRC mortality in the model would be a slight improvement in the cost-effectiveness of strategies with high yield of Lynch syndrome mutations.

Table 43: One-, five- and ten-year survival of colorectal cancer

Years since diagnosis	Male colon cancer	Female colon cancer	Male rectal cancer	Female rectal cancer	Model CRC
1	0.730	0.722	0.788	0.788	0.757
5	0.544	0.551	0.546	0.575	0.530
10	0.501	0.508	0.473	0.521	0.421

Source: Bowel cancer survival, Cancer Research UK.[1] Copyright © 2013, Cancer Research UK.

The hazard ratios for CRC mortality by age, compared to CRC mortality across all ages were estimated using net survival statistics from the ONS,[111] and are shown in *Table 44*. Details of calculations are given in Appendix 6 of Snowsill et al. 2014.[4]

Table 44: Hazard ratios for CRC mortality by age at diagnosis, compared to CRC mortality across all ages

Age group	Hazard ratio for CRC mortality		
	First year	Following four years	Thereafter
Under 70y	0.599	0.972	1
70–79y	0.956	0.966	1
80y and over	1.797	1.116	1

Metachronous colorectal cancer survival

As previously,[4] mortality due to metachronous colorectal cancer was modelled by adding the mortality rates for both the index and metachronous CRC as calculated above, assuming that mortality from the metachronous cancer would be no different to mortality from the index cancer for the same Dukes' stage (as assumed by, e.g., Mvundura et al. 2010[77] and Dinh et al. 2011[100]). The same approach is used independent of the Lynch syndrome status of the patient. As the mortality rates are dependent on the time since diagnosis in the model we keep track of time since diagnosis of the index cancer and the metachronous cancer.

Lynch syndrome colorectal cancer survival

As previously,[4] the model assumes improved survival for individuals with Lynch syndrome and local CRC compared to individuals with sporadic CRC. A hazard ratio of 0.57 is applied for Dukes' A and Dukes' B CRC if the simulated individual has Lynch syndrome, based on the study by Lin et al.[112] Survival for individuals with Dukes' C and Dukes' D CRC is equal for patients with and without Lynch syndrome, based on the study by Barnetson et al.[52]

5.1.2.2.4 Endometrial cancer

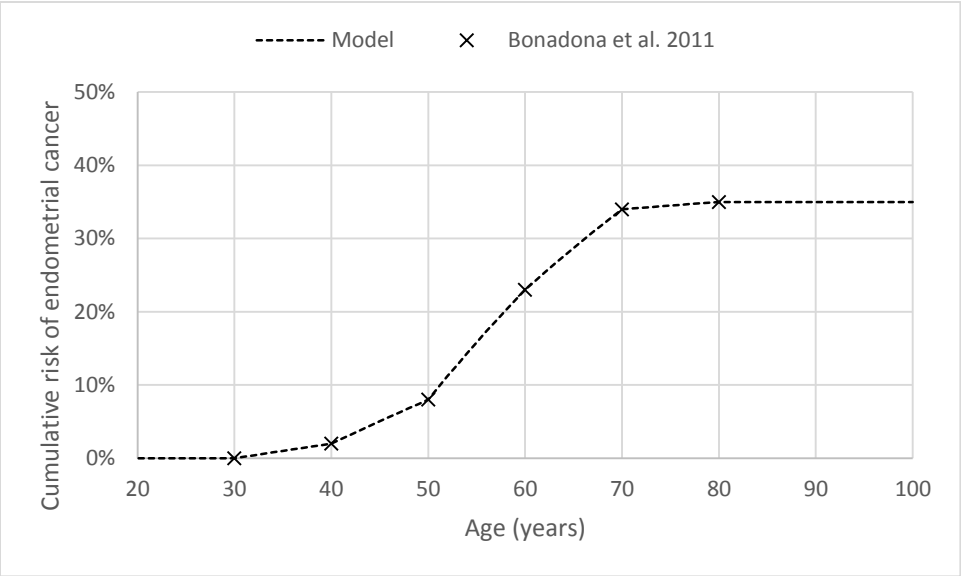
The lifetime risk of endometrial cancer in the general population is 1 in 41.[113] The lifetime risk in women with Lynch syndrome (in the absence of risk-reducing measures) is around 35%.[16] As previously,[4] endometrial cancer is only modelled for women with Lynch syndrome.

Endometrial cancer incidence

As previously[4] and as for colorectal cancer, the incidence rates for endometrial cancer in women with Lynch syndrome are estimated from the study by Bonadona et al.[16]

A piecewise constant hazard of endometrial cancer was used per decade of life to achieve the cumulative risk profile observed in the study by Bonadona et al., and it was assumed that the incidence of endometrial cancer would be zero after age 80. *Figure 24* shows the cumulative risk used in the model and the data from Bonadona et al. for reference.

Figure 24: Endometrial cancer incidence

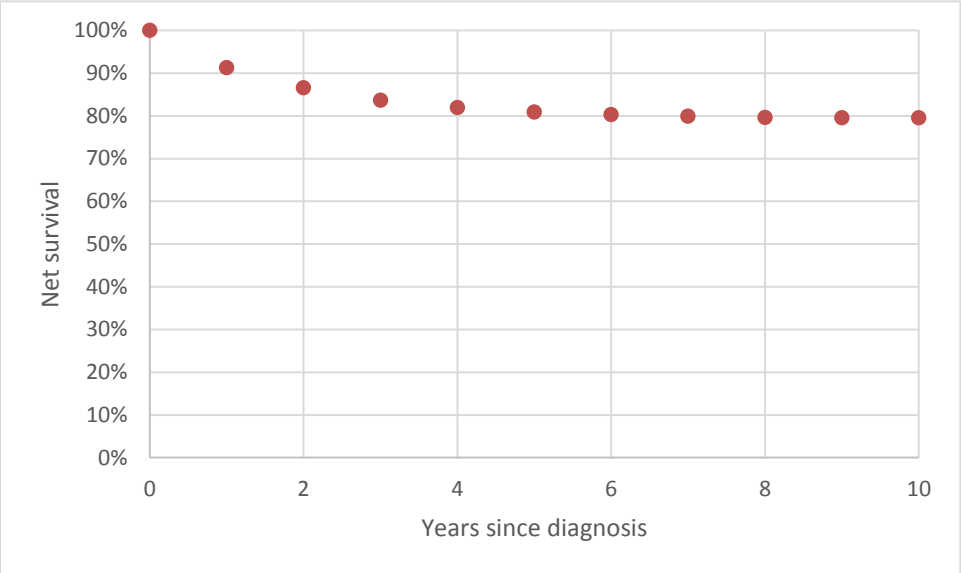


Gynaecological surveillance is not assumed to affect the incidence of endometrial cancer (see *Gynaecological surveillance*, *page 186*).

Endometrial cancer survival

Survival from uterine cancer in England and Wales has recently been estimated by the London School of Hygiene and Tropical Medicine and published by Cancer Research UK.[114]

Figure 25: Net survival for uterine cancer in England and Wales



Source: Cancer Research UK (2016) *Uterine cancer survival statistics*[114]

Endometrial cancer cases comprise the vast majority of uterine cancer, so these are expected to closely approximate survival of endometrial cancer.

The survival curve is used to estimate a piecewise constant rate of mortality for each year since diagnosis. After 10 years the rate of mortality is zero.

5.1.2.2.5 General mortality

Death from other causes was modelled by using mortality rates separately for men and women provided in life tables for England and Wales, 2008–2010,[115] adjusted to remove the proportion of mortality due to colorectal cancer, which was estimated by dividing the number of deaths from CRC in each age group by the total number of deaths in that age group from mortality data for England in 2010.[103] We did not adjust for mortality from endometrial cancer as this accounted for less than 1% of deaths in the general population (while CRC accounted for 2.8%) and we did not adjust for mortality from other Lynch syndrome associated cancers as these are not included in our model.

5.1.2.2.6 Surveillance pathways

Colorectal surveillance

Previously,[4] a colorectal surveillance pathway was modelled based on synthesis of a number of relevant recommendations,[43, 45, 116] which included biennial (every two years) colonoscopies for individuals diagnosed with Lynch syndrome.

The recently published guidelines from the European "Mallorca group" advocate an interval of 1–2 years between colonoscopies for individuals with Lynch syndrome.[19]

The evidence underpinning the effectiveness estimates for colonoscopic surveillance in the model is based on 3-yearly surveillance[117] (see *Colonoscopy*, *page 183*).

To avoid excessive inconsistency between the effectiveness evidence and the associated costs of colonoscopic surveillance, an interval of two years is modelled.

Colorectal cancer patients are assumed to also receive: a carcinoembryonic antigen (CEA) test every three months for two years and then every six months for a further three years; CT chest, abdomen and pelvis at 12 months and 24 months; colonoscopy at 12 months and then 5-yearly colonoscopy (unless diagnosed with Lynch syndrome).[4, 45, 116]

Gynaecological surveillance

The European Society of Medical Oncology guidelines recommend annual gynaecological examination, pelvic ultrasound, CA-125 analysis and aspiration biopsy, starting at age 30–35 years.[118] This is used as the basis for modelling gynaecological surveillance, with surveillance initially offered at age 35 (or immediately if the diagnosis of Lynch syndrome is after age 35) and continuing to age 70.

5.1.2.2.7 Colorectal surgery pathways

As previously,[4] colorectal surgical pathways are based on published guidelines[43] with input from clinical experts.

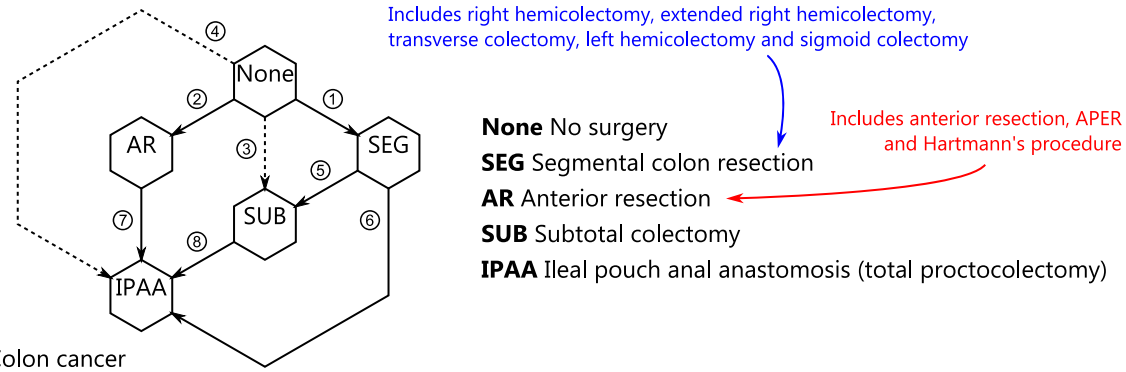
Patients undergo surgical management if they are diagnosed with CRC and the cancer is deemed to be operable (this includes surgery where intent is palliative rather than curative). We make the simplifying assumption that all patients diagnosed with CRC undergo surgical

management (over 75% of patients in the National Bowel Cancer Audit (2011)[101] were treated surgically). In each case, surgery would remove the bowel portion affected by the cancer, and in some cases additional portions, depending on previous surgery and whether Lynch syndrome had been diagnosed (see *Figure 26*, *page 177*, adapted from Maeda et al. 2010[99]). Our clinical expert advice is that in general, surgery for patients without Lynch syndrome tends to be conservative, without a risk-reducing element.

Clinical guidelines indicate that there is a place for more aggressive surgery, with a risk-reducing element, for patients known to have Lynch syndrome upon CRC diagnosis, in particular that "For patients with proximal tumours, colectomy and ileorectal anastomosis is most relevant".[43] Input from our clinical expert has suggested that this particular guidance would rarely be followed as it is from a low category of evidence (evidence obtained from expert committee reports or opinions or clinical experiences of respected authorities) and colonoscopic surveillance is deemed effective enough to negate the need for aggressive surgery. To resolve this disagreement we include a parameter in the model which defines the probability that more aggressive surgery would be used for Lynch syndrome patients which can be varied from 0 (ignore guidelines; surgical treatment not affected by Lynch diagnosis) to 1 (full adherence to guidelines; aggressive surgery always used). Previous analyses in Snowsill et al. (2014)[4] demonstrated that altering this parameter had minimal impact on cost-effectiveness and therefore sensitivity analyses on this parameter have not been repeated.

When surgery removes the rectum due to cancer in the rectum there are two common operations: anterior resections (AR) which preserve the anus and abdominoperineal excisions of the rectum (APER) which result in permanent stoma. We group these operations together and assume they are both as effective at preventing metachronous rectal cancer. Some patients would require a permanent stoma, which would affect HRQL and costs. Rather than modelling this on an individual patient basis we assume an average effect across all patients.

Figure 26: Surgical management pathways for colorectal cancer



① Colon cancer
② Rectal cancer
③ Synchronous colon cancer or polyps in distant sections of colon or aggressive management of colon cancer in Lynch syndrome patient
④ Synchronous colon and rectal cancer or synchronous rectal cancer and colonic polyps or aggressive management of rectal cancer in Lynch syndrome patient
⑤/⑦ Metachronous colon cancer
⑥/⑧ Metachronous rectal cancer

Any subsequent surgery depends on the location of the CRC, the nature of previous surgery and whether the patient has been diagnosed with Lynch syndrome (unless the parameter described above is 0) (see *Table 45* and *Table 46*).

Table 45: Probability of different surgery types for colon cancer patients not diagnosed with Lynch syndrome

Previous surgery	Segmental resection	Subtotal colectomy	Anterior resection	Proctocolectomy	Source
None	96%[a]	4%[b]	0%	0%	NHS Bowel Cancer Audit report 2011[101]
Segmental resection	0%	100%	0%	0%	Assumption
Subtotal colectomy	N/A	N/A	N/A	N/A	Assumption
Anterior resection	0%	0%	0%	100%	Assumption
Proctocolectomy	N/A	N/A	N/A	N/A	Assumption

Notes: N/A because subtotal colectomy and proctocolectomy are assumed to completely eliminate the risk of colon cancer
[a] 8,850 colon cancer patients underwent right hemicolectomy (n=6,627), transverse colectomy (n=86), left hemicolectomy (n=978) or sigmoid colectomy (n=1,159)[101]
[b] 325 colon cancer patients underwent total or subtotal colectomy[101]

Table 46: Probability of different surgery types for rectal cancer patients not diagnosed with Lynch syndrome

Previous surgery	Segmental resection	Subtotal colectomy	Anterior resection	Proctocolectomy	Source
None	0%	0%	98%[a]	2%[b]	NHS Bowel Cancer Audit report 2011[101]
Segmental resection	0%	0%	0%	100%	Assumption
Subtotal colectomy	0%	0%	0%	100%	Assumption
Anterior resection	N/A	N/A	N/A	N/A	Assumption
Proctocolectomy	N/A	N/A	N/A	N/A	Assumption

Notes: N/A because anterior resection and proctocolectomy are assumed to completely eliminate the risk of rectal cancer
[a] 4,341 rectal cancer patients underwent anterior resection (n=2,890), APER (n=1,139) or Hartmann procedure (n=312)[101]
[b] 82 rectal cancer patients underwent total or subtotal colectomy[101]

Surgery distributions for CRC patients diagnosed with Lynch syndrome are adjusted by the parameter representing the probability of aggressive surgery. If we denote this probability as p then for colon cancer patients:

$$\Pr(\text{SEG}|\text{LS diagnosed}) = (1-p)\Pr(\text{SEG}|\text{LS not diagnosed})$$
$$\Pr(\text{SUB}|\text{LS diagnosed}) = 1 - \Pr(\text{SEG}|\text{LS diagnosed})$$

Where SEG is segmental resection and SUB is subtotal colectomy. For rectal cancer patients:

$$\Pr(\text{AR|LS diagnosed}) = (1-p)\Pr(\text{AR|LS not diagnosed})$$
$$\Pr(\text{IPAA|LS diagnosed}) = 1 - \Pr(\text{AR|LS diagnosed})$$

In the base case, this parameter is set to 0, for the following reasons:

- The clinical guidelines (e.g., the BSG/ACPBGI guidelines[43]) are not based on high quality evidence;
- The model does not incorporate any utility decrement for more aggressive surgery, even though function can be affected (see *Colorectal cancer treatment*, *page 195*);
- The model does not incorporate excess surgical mortality from more aggressive surgery.

5.1.2.2.8 Aspirin chemoprevention

Aspirin has been shown in observational studies to reduce colorectal cancer burden[119] and the CAPP2 randomised controlled trial in individuals with Lynch syndrome mutations demonstrated a protective effect from aspirin against colorectal cancer and other Lynch syndrome associated cancers in individuals completing the protocol.

As the balance of risks and benefits is favourable for prescribing regular aspirin to individuals with Lynch syndrome (unless contraindicated) – and aspirin use in this context is highly likely to be cost-effective (and patients may also purchase over the counter if not prescribed) – it was judged that aspirin chemoprevention should be included as part of the base case analysis.

It should be noted that at present individuals with Lynch syndrome are most likely to receive aspirin as part of the ongoing CaPP3 dosing study rather than by being directly prescribed.

A scenario analysis is also conducted in which aspirin is not prescribed.

5.1.3 Perspective, time horizon and discounting

Costs are included from a NHS and personal social services (PSS) perspective.

The perspective on health outcomes is all direct health effects on patients, which in this case includes individuals newly diagnosed with colorectal cancer (probands) and their blood relatives who may be at risk of Lynch syndrome.

A lifetime time horizon is used, with a maximum age of 100 modelled.

Costs and QALYs are discounted at 3.5% per annum (unless otherwise stated). Life years and natural outcomes (e.g., number of colorectal cancers) are not discounted (unless otherwise stated).

5.1.4 Model parameters

A summary table of all model parameters is provided in *Appendix 5*.

5.1.4.1 Treatment effectiveness and extrapolation

5.1.4.1.1 Diagnostic performance

Estimates of test accuracy (sensitivity and specificity) were taken from available literature, identified via the diagnostic accuracy and cost-effectiveness literature reviews reported in *Section 2* and *Section 4*.

In Snowsill et al. (2014), test accuracy for IHC and MSI was taken from the EGAPP review, reported by Palomaki et al.[39] The EGAPP review synthesised studies with very different input populations, not all relevant to this review. In the current model, population based studies identified in *Section 2* (Barnetson et al. 2006, Southey et al. 2005, Poynter et al. 2008, Limburg et al. 2011)[31, 52-54] and use these to produce estimates of test accuracy. *Section 2* (*page 67*) explains that a meta-analysis of the included studies is not conducted, due to the heterogeneity of the studies. In particular, though they are population based studies, Barnetson et al., Southey et al., and Limburg et al. all used age limits on their input population, which will influence the prevalence of these populations and potentially the sensitivity and specificity of the tests.

The study results were synthesised using the multilevel mixed-effects logistic regression command 'melogit' in Stata/SE version 14.1 (StataCorp LP, Texas, USA). The code for this analysis is presented in *Appendix 5*. The results of this analysis (with 95% CIs), plus the EGAPP results, are presented in *Table 47*. The values from Snowsill et al. (2014) lie well within the confidence intervals of the new estimates, though the new point estimates for sensitivity appear higher and specificity slightly lower than reported by the EGAPP review.

Due to the low number of studies (three for MSI, two for IHC), it was not possible to estimate the correlation between true sensitivity and specificity across studies, so this parameter was removed from estimation (i.e., independence of random effects was assumed).

As indicated in *Section 2*, this synthesis may not be entirely appropriate to determine the accuracy for the purposes of review. This is due to the small number of studies, the variation in methodology (e.g., heterogeneity in reference standard) and the heterogeneity of accuracy estimates (e.g., Poynter et al.[31] predict much higher sensitivity and lower specificity for MSI than the other two studies). The small number of studies means it is not possible to adequately explore sources of heterogeneity, and it is also not possible to assess the likelihood of publication bias (e.g., using a funnel plot). However, the confidence intervals are sufficiently wide that for modelling purposes a range likely to include the true values can be implemented in univariate sensitivity analyses.

Accuracy results for *BRAF* V600E and *MLH1* promoter methylation testing are taken from the recent technical review by Ladabaum et al. (2015),[3] which was identified from the searches run in the cost-effectiveness review (*Section 4.2.1*). This technical review synthesised 14 studies reporting *MLH1* promoter methylation and 11 studies reporting *BRAF* accuracy. These studies included a variety of prior tests, including MSI and IHC. This updates the values from Snowsill et al. (2014), which were based on very few studies.

Accuracy for genetic testing in probands and relatives is unchanged from Snowsill et al. (2014) and is taken from published literature. Diagnostic genetic testing for *PMS2* is assumed to have lower sensitivity than testing for the other genes, given the greater complexity of molecular analysis of this gene (which results in a lower pick-up rate) and the

greater difficulties in interpreting mutations (when found) as pathogenic (given the lower penetrance of mutations in *PMS2*).[5, 42]

Table 47: Test accuracy parameters

Test	Parameter	Base case MSI= MSI-H (95% CI)	Scenario analysis MSI=MSI-L and MSI-H (95% CI)	Source	Snowsill et al. (2014) base case	Source
MSI	Sensitivity	0.913 (0.426-0.993)	0.973 (0.893-0.994)	Barnetson, 2006[52] Poynter, 2008[31] Southey, 2005[54]	*MLH1*, *MSH2* 0.89 *MSH6*, *PMS2* 0.77	Palomaki, 2009[39]
	Specificity	0.837 (0.638-0.937)	0.596 (0.304-0.833)		0.902	
IHC	Sensitivity	0.962 (0.694-0.996)	*	Limburg, 2011[53] Southey 2005[54]	0.77	
	Specificity	0.884 (0.790-0.940)	*		0.888	
BRAF	Sensitivity	0.96 (0.60-0.99)	*	Ladabaum, 2015[3]	1.00	Domingo, 2004[120]
	Specificity	0.76 (0.60-0.87)	*		Following MSI: 0.40 Following IHC: 0.69	Domingo, 2004[120] Palomaki, 2009[39]
MLH1 promoter methylation	Sensitivity	0.94 (0.79-0.98)	*	Ladabaum, 2015[3]	0.93	Bouzourene, 2010[121] Chang, 2010[122]
	Specificity	0.75 (0.59-0.86)	*		0.83	
Diagnostic genetic testing for probands	Sensitivity	*MLH1*, *MSH2*, *MSH6* 0.90 *PMS2* 0.67		Dinh, 2011[100] Senter, 2008[123]		
	Specificity	0.997				
Predictive genetic testing for relatives	Sensitivity	1.00		Assumed[4]		
	Specificity	1.00				

Key: * Same as base case

One other important component of the effectiveness of a strategy is the acceptance rate of the tests. Acceptance rates of diagnostic tests, and their sources, are reported in *Table 48*. These remain unchanged from Snowsill et al. (2014), with the exception of the acceptance of counselling and genetic testing for relatives, which has been updated to use UK data from the Manchester Lynch syndrome cancer registry as reported in Barrow (2015).[84] Previously these values were taken from Palomaki et al. (2009),[39] and were not UK specific.

Table 48: Rates of acceptance of diagnostic tests and genetic counselling.

Test	Proband/Relative	Acceptance rate	Original source
MSI	Proband	100%	Ramsey et al. 2003[76] confirmed by expert IMF in Snowsill et al. (2014)
IHC	Proband	100%	Assumed
BRAF V600E	Proband	100%	Assumed
MLH1 promoter hypermethylation	Proband	100%	Assumed
Genetic test following counselling (proband)	Proband	90%	Ladabaum et al. 2011[78]
Genetic counselling (proband)	Proband	92.5%	Clinical experts (IMF) gave range 90-95% in Snowsill et al. (2014)[4]
Genetic test following counselling (relative)	Relative	77%	Calculated from Manchester Familial colorectal cancer registry data reported in Barrow (2015)[84]
Genetic counselling (relative)	Relative	78%	Calculated from Manchester Familial colorectal cancer registry data reported in Barrow (2015)[84]

5.1.4.1.2 Surveillance

Colonoscopy

Colonoscopy is assumed to lead to improved health outcomes by reducing colorectal cancer incidence (as adenomas are identified and removed which could have become adenocarcinomas) and by improving the cancer stage distribution on diagnosis (i.e., catching the cancer earlier), which leads to improved survival.

The model assumes that the majority of patients diagnosed with Lynch syndrome will be offered and will accept surveillance colonoscopies.

Data of 591 individuals from the Manchester Familial Colorectal Cancer Registry, as reported in Barrow (2015)[84] is used to estimate acceptance of relatives diagnosed with Lynch syndrome. We assume that this rate will be the same for probands as for relatives, as in Snowsill et al. (2014) the acceptance of surveillance in probands was the same or higher than that of relatives. Previously these estimates were taken from Ladabaum et al. (2011),[78] which was a US population and may not reflect the acceptance rate of the UK population. The current values appear to suggest a higher acceptance rate than previously modelled. However, as these values are taken from only one UK institution and acceptance of

surveillance has been previously shown to be an influential parameter on cost-effectiveness, we examine the impact it has via sensitivity analyses.

Table 49: Initial rates of acceptance of Lynch syndrome surveillance for colorectal cancer

Patient characteristic	Initial acceptance of surveillance[84]	Value in Snowsill et al. (2014) (based on Ladabaum et al. 2011[78])
Proband tested LS mutation positive	97%	80%
Proband LS assumed	70%	70%
Relative tested LS mutation positive	97%	80%
Relative LS assumed	70%	50%

Colorectal cancer incidence

Previously,[4] event times were extracted from Figure 1 of Järvinen et al. (2000)[117] and used in a Cox proportional hazards regression to estimate the effectiveness of colonoscopy in reducing colorectal cancer incidence. The resulting hazard ratio estimate was 0.387 (95% CI, 0.169 to 0.885).

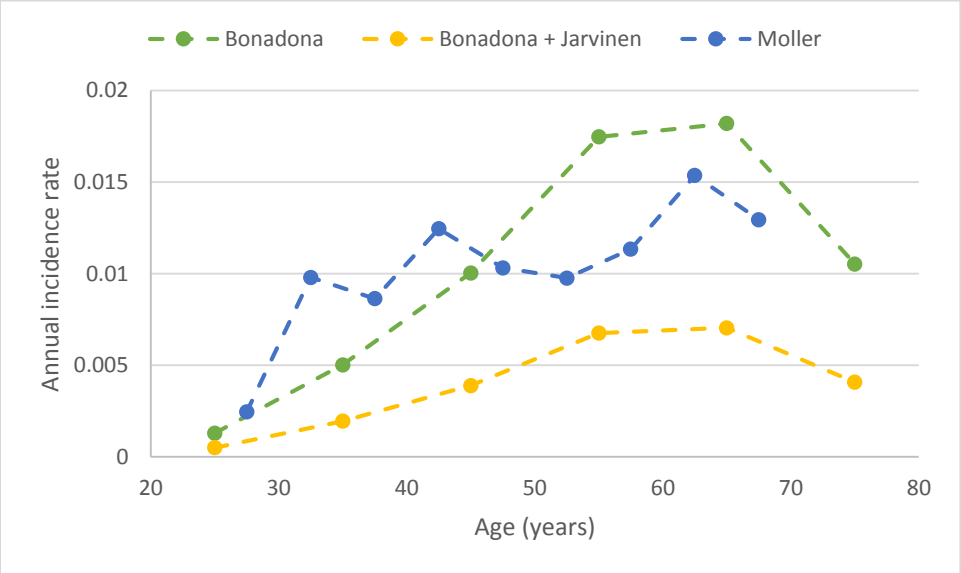
Subsequently, other evidence has also been collected relating to this question.

The American Gastroenterological Association published a technical review on the diagnosis and management of Lynch syndrome in 2015, which identified five observational studies (including Järvinen et al. 2000) which allowed estimation of the effectiveness of colonoscopy in reducing colorectal cancer incidence.[3] The pooled effect estimate (odds ratio) was 0.23 (95% CI, 0.13 to 0.41).

Møller et al. (2015)[5] report on the cancer risks in a prospectively identified population of Lynch syndrome carriers in which surveillance is widespread. This study includes 1942 Lynch syndrome carriers from across Europe (and Australia), and 195 of these from the UK. The authors found high rates of colorectal cancer in spite of regular surveillance.

Figure 27 illustrates the contradictory nature of the evidence. “Bonadona” and “Moller” are the estimated incidence rates in the studies, while “Bonadona + Jarvinen” is a counterfactual incidence rate profile obtained by applying the hazard ratio 0.387 to the “Bonadona” data. If all these studies were conducted without bias and in similar populations and with enough statistical power one would expect “Moller” to closely align to “Bonadona + Jarvinen” rather than “Bonadona”.

Figure 27: Comparison of colorectal cancer incidence rates in the absence of surveillance (Bonadona) and with surveillance (Møller)



Notes: Assumes 41% *MLH1*, 44% *MSH2* and 16% *MSH6* (figures do not sum to 100% due to rounding)
Sources: Bonadona et al. 2011[16] and Møller et al. 2015[5]

There are a number of potential reasons why the results of Møller et al. might not confirm the effectiveness of colonoscopy observed in Järvinen et al.:

- Limited statistical power in both the Bonadona and Møller studies (i.e., random chance);
- Naïve comparison of results from Bonadona and Møller studies with no adjustment for differences in populations (other than for gene distribution and age);
- Biased effect estimate in Järvinen et al. (2000).

It is difficult to assess the similarity of the Bonadona and Møller patient populations. The population in Bonadona et al. seems to have a more even gender balance while the population in Møller et al. has somewhat more women. It is possible that there are differences in the proportion of patients with clearly pathogenic mutations – Bonadona et al. included 7% (35/537) families with VUS, while Møller et al. only included patients with mutations judged pathogenic by their reporting centre (although 31% of patients had mutations not reported on the Leiden Open Variant Database by October 2015).

The two main sources of potential bias in the Järvinen et al. study are:

- Confounding due to self-selection of intervention group (i.e., participants chose whether or not to receive the intervention) – this would be expected to exaggerate the effectiveness estimate since participants choosing to receive surveillance are more likely to have better health behaviours and other factors;
- Confounding due to treatment switching (i.e., participants who initially declined surveillance later opted into surveillance) – this would be expected to attenuate the effectiveness estimate.

Furthermore, the results from Järvinen et al. may not generalise to current surveillance in the NHS due to developments in technology, or to differences in service delivery and behaviour which could result in a different distribution of screening intervals.

However, in the absence of compelling alternative effectiveness estimates, we continue to use the hazard ratio of 0.387 from Järvinen et al.

In a worst case scenario analysis it is assumed that surveillance does not reduce colorectal cancer incidence, i.e., a hazard ratio of 1 is used.

Stage on diagnosis

As previously,[4] the stage on diagnosis is assumed to be dependent only on whether an individual has been offered and accepted Lynch syndrome surveillance colonoscopies. Whether the individual has a Lynch syndrome mutation, their previous history of cancer, their sex and age were not modelled as affecting the stage distribution.

The stage on diagnosis for individuals not accepting surveillance was estimated from national data from England.

In England in 2012, 11% of colorectal cancers were not staged (or the stage was not recorded).[124] The National Cancer Intelligence Network (NCIN) used multiple imputation methods, based on patient and cancer characteristics which were recorded, to estimate the stage distribution for the unstaged patients (*Table 50*). When this stage distribution is combined with the data for patients whose cancers were staged, the resulting distribution is 17.6 : 27.0 : 29.5 : 25.9. Previously a distribution of 16.4 : 31.7 : 27.1 : 24.8 was estimated from 2009/10 data by excluding patients whose cancer stage was not recorded.[4] The effect of the change is to reduce the number of patients with Stage II colorectal cancer and increase the number of patients with other stages.

Table 50: Stage distribution of colorectal cancers in England

Stage	Observed data for all patients		Imputed stage distribution of "Unknown"		Combined data	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
Stage I	5,255	(15.5%)	734	(2.16%)	5,989	(17.6%)
Stage II	8,402	(24.7%)	768	(2.26%)	9,170	(27.0%)
Stage III	9,258	(27.2%)	778	(2.29%)	10,036	(29.5%)
Stage IV	7,351	(21.6%)	1,465	(4.31%)	8,816	(25.9%)
Unknown	3,745	(11.0%)				
Total	34,011	(100.0%)	3,745	(11.02%)	34,011	(100%)

Source: Calculated from Table 1 and Table 4 of *National Cancer Intelligence Network, Cancer Survival in England by stage (2014)*[124]

For individuals accepting Lynch syndrome surveillance colonoscopies, a stage distribution of 68.6 : 10.5 : 12.8 : 8.1 was used as previously,[4] based on data from Mecklin et al. (2007).[125]

Gynaecological surveillance

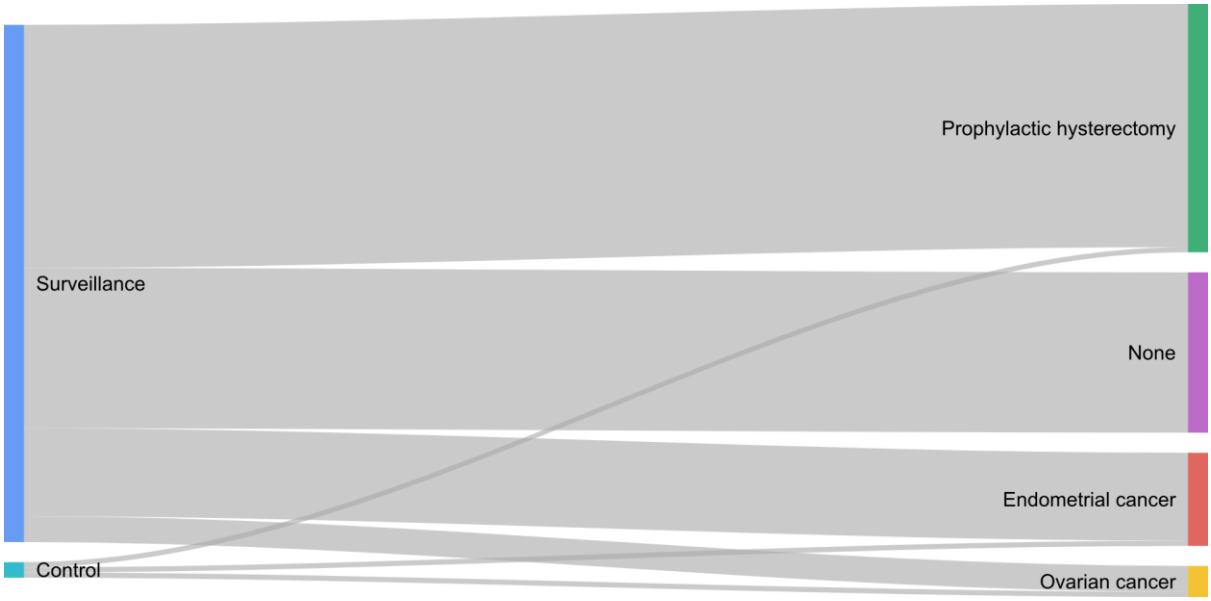
A literature review was conducted previously of the effectiveness of surveillance for endometrial and ovarian cancer.[4] Conceptually, surveillance was expected to reduce the

incidence of gynaecological cancers by the detection of pre-malignancies, which are either individually removed, or which prompt hysterectomy. Malignancies identified during surveillance were also expected to have a more favourable stage profile.

No experimental studies (e.g., RCTs) were identified, but three non-experimental studies were identified.[126-128] Two of these studies[127, 128] considered cohorts of women with Lynch syndrome eligible for gynaecological surveillance and compared patients receiving surveillance with patients refusing or not receiving surveillance during the study (see *Figure 28* and *Figure 29*). These study designs could give an estimate of the effectiveness of surveillance in reducing incidence of gynaecological cancer, but they are both at high risk of bias due to potential confounding factors between the groups and neither can give satisfactory effect size estimates due to limited control group sizes and the limited number of events. The limited number of events also means that these studies are not informative for the stage distribution of gynaecological cancers.

Figure 28: Patient flow diagram for Dove-Edwin et al. 2002

Figure 29: Patient flow diagram for Jarvinen et al. 2009



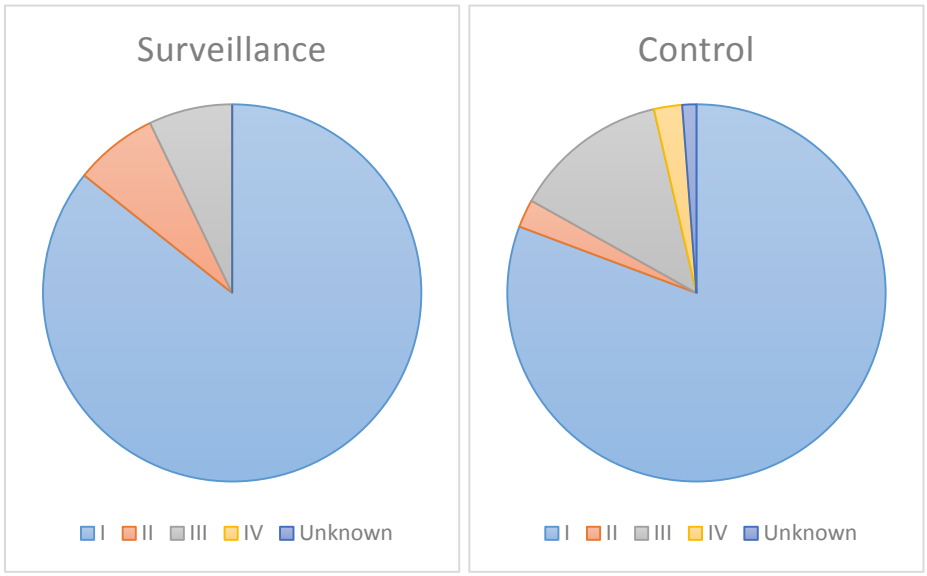
The study by Renkonen-Sinisalo et al. (2007)[126] by contrast considers patients before and after institution of a surveillance programme. The outcomes of 385 women with Lynch syndrome mutations were compared to the outcomes of 83 women with Lynch syndrome mutations who were affected by endometrial cancer before surveillance was instituted (*Figure 30*).

Figure 30: Patient flow diagram for Renkonen-Sinisalo et al. 2007

Key: EC, endometrial cancer

This study design cannot be used to estimate the effect of surveillance on cancer incidence, but it can be used to estimate the impact of surveillance on the stage of cancer at diagnosis (*Figure 31*).

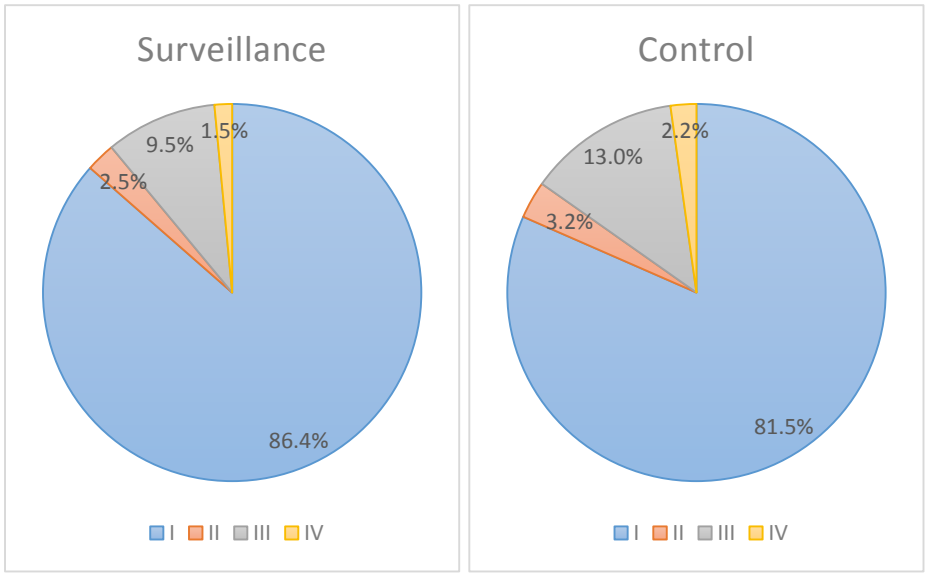
Figure 31: FIGO stage distribution of endometrial cancers in Renkonen-Sinisalo et al. 2007



Key: FIGO, International Federation of Gynaecology and Obstetrics

An ordered logistic regression was used to estimate the impact of surveillance. The regression coefficient for surveillance was not statistically significant ($z = -0.45$, $p = 0.651$), but was suggestive that surveillance could improve the stage distribution. Predicted stage distributions from the regression are shown in *Figure 32*.

Figure 32: Predicted stage distribution based on results of Renkonen-Sinisalo et al. 2007



These stage distributions were not used directly, but were instead used to estimate a hazard ratio for survival from endometrial cancer.

Lewin et al. (2010)[106] estimated the 5-year survival from endometrial cancer according to stage at diagnosis (*Table 51*). The weighted average 5-year survival for patients in the surveillance and control groups were estimated as 83.4% and 81.8% respectively, from which a hazard ratio of 0.898 was derived.

Table 51: 5-year survival from endometrial cancer according to stage at diagnosis

FIGO (1998) stage	5-year survival (%)
I	87.8
II	76.2
III	55.3
IV	21.1

Key: FIGO, International Federation of Gynaecology and Obstetrics
Source: Calculated from results presented by Lewin et al. 2010[106]

Owing to the significant uncertainty in this hazard ratio, a scenario analysis is included in which there is no survival benefit (i.e., no benefit at all) from gynaecological surveillance.

It should be noted that the women in this study were offered surveillance with two or three year intervals, whereas current European guidelines propose annual surveillance,[118] so it is possible that a study of annual surveillance could find greater effectiveness than what is modelled.

5.1.4.1.3 Surgery

5.1.4.1.4 Chemoprevention

There is evidence from the CAPP2 randomised controlled trial that aspirin reduces the incidence of cancer in individuals with Lynch syndrome mutations.[28] In this study participants were randomised to receive aspirin (600 mg enteric coated aspirin daily) or aspirin placebo (alongside resistant starch or starch placebo in a two-by-two factorial design) for up to four years.

Intention-to-treat and per-protocol analyses were conducted. Per-protocol analyses considered patients who continued to take aspirin (or aspirin placebo) for at least two years.

Analyses were conducted using Cox proportional hazards (to obtain a hazard ratio for incidence of first cancer) and Poisson regression (to obtain an incidence rate ratio accounting for multiple cancers within each individual).

A total of 861 participants were randomised to aspirin (N = 427) or aspirin placebo (N = 434).

At the time of publication the mean follow-up was 55.7 months (4.6 years) with maximum follow-up 128.0 months (10.7 years).

The intention-to-treat proportional hazards analysis showed a reduction in colorectal cancer incidence (HR 0.63; 95% CI, 0.35 to 1.13) but did not reach statistical significance (P = 0.12). A number of other analyses did reach statistical significance and the authors concluded that aspirin is effective in reducing the risk of Lynch syndrome cancers (*Table 52*).

Table 52: Summary of key results from CAPP2 trial

Analysis	Hazard ratio (95% CI)	Incidence rate ratio (95% CI)
Intention-to-treat		
CRC	0.63 (0.35–1.13)	0.56 (0.32–0.99)
LS cancers except CRC	0.63 (0.34–1.19)	0.63 (0.34–1.16)
All LS cancers	0.65 (0.42–1.00)	0.59 (0.39–0.90)
Per-protocol[a]		
CRC	0.41 (0.19–0.86)	0.37 (0.18–0.78)
LS cancers except CRC	0.47 (0.21–1.06)	0.49 (0.23–1.05)
All LS cancers	0.45 (0.26–0.79)	0.42 (0.25–0.72)

Notes: [a] Hazard ratios and incidence rate ratios refer to patients taking aspirin for ≥2 years vs. patients taking aspirin placebo for ≥2 years
Source: Adapted from Table 2 of Burn et al. 2011[28]

Not all patients recruited to the trial (N = 1071) were randomised to aspirin or aspirin placebo; 134 were ineligible to receive aspirin or withdrew before treatment, and 76 participants requested not to receive aspirin. On this basis it was estimated that 80.4% of patients would be offered and accept aspirin chemoprevention upon diagnosis of Lynch syndrome.

The majority (59%) of participants randomised to aspirin or aspirin placebo were treated for two years or longer. It was assumed that 59% of individuals accepting aspirin chemoprevention would receive aspirin for four years and would have reduced incidence of CRC and EC (incidence rate ratios of 0.37 and 0.49 respectively), while the remaining individuals would receive no aspirin and see no change in their incidence rates.

Since the estimates are taken from a study with maximum follow-up just over 10 years, it was assumed conservatively that the duration of effect would be 10 years.

5.1.4.2 Health related quality of life

Systematic searches were conducted for utilities associated with CRC, endometrial cancer and prophylactic hysterectomy. The literature searches for utility studies were conducted in MEDLINE (Ovid) and Embase (Ovid). Searches comprised of population terms for hysterectomy and salpingo-oophorectomy, colorectal cancer and endometrial cancer, combined with relevant utility terminology. The searches for colorectal cancer utility studies were date limited from 2005 to date and literature published prior to 2005 was identified using Snowsill et al. (2014). The hysterectomy and salpingo-oophorectomy and the endometrial cancer searches were not date limited. Searches for each population group were conducted separately, then combined and de-duplicated using Endnote X7. The full search strategies and the number of hits per database and in total are detailed in *Appendix 1*.

The searches were screened (first by title and abstract, then full text) by one reviewer (NH), who also carried out data extraction and assessed the studies for suitability in parameterising the model.

5.1.4.2.1 Colorectal cancer

Twelve full texts reporting CRC and related utilities were identified: six of which reported the effect of CRC on utility estimates and six of which reported the effect of CRC treatment on utilities. An overview of these studies is given in *Table 53*. Several studies reported multiple utility measures, but only those that are most relevant to the review are presented here.

Some studies reported a comparison between colorectal cancer quality of life and general population quality of life, and the difference did not appear statistically significant for measures of general health. When studies reported results by stage, there was some evidence that suggested Stage IV would result in a reduction in quality of life.[129-131]

The findings of these results were consistent with what has been previously modelled (*Table 54*). As such the base case remains unchanged Snowsill et al. (2014), with no disutility assumed for individuals diagnosed with Dukes' A, B or C and a disutility of 0.13 for individuals diagnosed with Dukes' D.[132, 133] A scenario analysis is used to investigate the possibility of an increased disutility associated with CRC, using the figures from Ness et al. (1999),[134] as reported previously in Snowsill et al. (2014).[4] This is consistent with other cost-effectiveness analyses, which have used the same sources.[135-138]

Table 53: Colorectal cancer studies reporting health related quality of life

Study	Population (n)	Method	Utility measure	Results	Advantages	Limitations
Farkkila 2013[139]	508 Finnish CRC patients	Cross sectional observational survey	EQ-5D	EQ-5D change from standardised gen population: Primary treatment (local disease 0-6m after diagnosis) -0.033 Rehab (local 6-18m) 0.064 Remission (local >18 months) 0.046 Metastatic disease (receive treatment) -0.005 Palliative care -0.119	EQ-5D, study did not influence treatment decisions Uses UK TTO tariff for EQ-5D	States based on time since diagnosis, not stage at diagnosis. Non UK based Disease severity of non-responders unknown
Hall 2015[140]	128 (down to 97 at last follow up) CRC patients(within 6 months of diagnosis) at two NHS trusts (Leeds teaching hospital trust and Calderdale & Huddersfield NHS foundation trust	Hospital based survey. Routine data collection through us of online systems at 3 time points	EQ-5D	<6months post diagnosis:0.765 (GP 0.793) 9 months: 0.802 (GP 0.793) 15 months 0.812 (GP 0.794) At no time point was the difference in QoL from GP statistically significant	EQ-5D, UK NHS	Only two trusts Aim of analysis was to look at costs, QoL All pts had to have internet access Only 1 Dukes' D patient Dukes stage unknown for 75 responders Utility by Dukes stage not reported
Hung 2013[129]	134 colorectal cancer patients in Taiwan	Descriptive and longitudinal cohort study	FACT-G	FACT-G Stage IV vs. 1 -15.16 (7.34) III vs. I 1.10 (4.31) II vs. I -0.44 (4.02)	CRC patients, difference by stage reported – all stages represented	FACT-G not EQ-5D. No comparison to population. Taiwan population Small sample size for some stages

Mhaidat 2014[130]	74 colorectal cancer patients in Jordan	Cross-sectional study	EORTC QLQ-C30, EORTC QLC-CR29	EORTC QLQ-C30 GHS not statistically influenced by age median 66.67 for all Median GHS by stage (range) I 70.83 (41.67-100), II 75 (41.67-100) III 66.67 (0-100) IV 50 (16.67-91.67)	CRC patients, all stages represented and utilities reported by stage	EORTC not EQ-5D, small sample size (14-27 pts in each stage) Jordan population
Stein 2014[141]	74 mCRC patients in UK and Netherlands	Observational, non-interventional, cross-sectional single visit study	EQ-5D-3L	Pre-progression 0.741+/-0.230 Post-progression 0.731 +/-0.292	Includes UK patients EQ-5D	mCRC patients Small sample size Utilities by CRC stage not reported
Wong 2013[131]	381 CRC patients at Queen Mary Hospital, Hong Kong, (subgroup of colorectal neoplasms patients)	Cross sectional study	Chinese version of SF-12v2 and SF-6D	SF-6D Mean, by stage: I 0.831 +/- 0.14 II 0.858+/-0.12 III 0.817 +/- 0.13 IV 0.732+/-0.15	Large sample Results by stage	Chinese population and utility measure One institution No comparison with GP for SF-6D

Key: CRC, colorectal cancer; mCRC, metastatic CRC; GP, general population

Table 54: Disutilities associated with CRC

CRC stage	Base case		Sensitivity analysis	
	Disutility	**Based on study**	**Disutility**	**Based on study**
Dukes' A	0.00	Ramsey 2000[132]	0.11	Ness 1999[134]
Dukes' B	0.00	Ramsey 2000[132]	0.23	Ness 1999[134]
Dukes' C	0.00	Ramsey 2000[132]	0.26	Ness 1999[134]
Dukes' D	0.13	Mittmann 2009[133]	0.60	Ness 1999[134]

Source: Snowsill et al. (2014)[4]

Colorectal cancer treatment

Four studies[142-145] all reported that more extensive colorectal surgery does not appear to impact the quality of life in CRC patients with familial cancer syndromes, including patients with Lynch syndrome. These studies report findings from a variety of countries (Netherlands, USA, Australia and New Zealand), though none are UK based. The quality of life questionnaires used in these studies are EORTC QLQ C-30 or SF-36, so the presented utility estimates cannot be directly compared to each other or to the EQ-5D, however the message appears consistent that type of surgery does not adversely impact quality of life in CRC patients. These findings agree with those reported in Snowsill et al. (2014) and as such, in our base case we assume no disutility for more extensive surgeries.

One study, Hornbrook et al. (2011),[146] presented SF-6D results for US CRC survivors with or without ostomies. They indicated that disutility from ostomies could be explained by other patient characteristics. No other studies reported HRQL results for CRC survivors according to whether they had received ostomies or not. We therefore assume no disutility for patients with ostomies compared to those without in our base case.

One study, Thong et al. (2011),[147] presented SF-36 scores for CRC patients in the Netherlands who were receiving either radiotherapy or radiotherapy and chemotherapy. Their results suggested the addition of chemotherapy did not significantly impact quality of life. No other studies reported HRQL results for CRC survivors according to whether they were receiving chemotherapy or not. We therefore assume no disutility according to treatment in our base case.

Colorectal cancer prevention

One study (Niv et al. 2012),[148] presented SF-36 results for 100 individuals undergoing colonoscopy for various reasons and indications, including surveillance for CRC (21 surveillance following CRC, 13 family history of CRC), in Israel. General health was found to be comparable before and after (both immediate and 1 month after) colonoscopy for non-inflammatory bowel disease patients (n=88), including those receiving surveillance for CRC, and no single component of the SF-36 was found to be statistically significantly different after colonoscopy. No other studies were identified that reported HRQL for individuals receiving colonoscopy. Therefore no disutility for asymptomatic individuals resulting from colonoscopy is assumed in the PenTAG base case.

5.1.4.2.2 Endometrial cancer

Five studies were identified that reported endometrial cancer QoL estimates[149-153]; two reported the PORTEC2 phase III randomised control trial at multiple time points,[149, 153] which had the largest population (427 at baseline, 80 at 10 year follow up), longest follow up (maximum 10 years) and compared the quality of life estimates for endometrial cancer to general population estimates. This trial included Stage 1, high-risk endometrial cancer and reported HRQoL from the EORTC QLQ-C30. One study (Nout et al., 2012)[149] reported the comparison with the general population and demonstrated an equal or improved HRQoL as time progressed after diagnosis and initial treatment (TAH-BSO).[149] Though PORTEC2 was based in the Netherlands, this finding was supported by the results of the other three studies (Ferrandina et al. 2014; Hildebrandt et al., 2014; Goker et al., 2011),[150-152] which were based in Italy,[152] Germany[150] and Turkey.[151]

We therefore used the PORTEC2 trial as our source of disutility for endometrial cancer compare to general population. We mapped the EORTC QLQ-C30 results to the EQ-5D using the algorithm provided by Longworth et al. (2014),[154] which has been validated by Doble and Lorgelly (2015).[155] Longworth et al. (2014) created an algorithm to map from the EORTC QLQ-C30 to the EQ-5D, based on 771 patients with multiple myeloma (VISTA trial), breast cancer and lung cancer (Vancouver Cancer clinic). Their work was funded by the UK Medical Research Council and as such was conducted from a UK perspective, though the EORTC QLQ-C30 data came from international sources. The algorithm provided estimates for each of the dimensions of the EQ-5D that can then be transformed into a utility using results from Dolan (1997),[156] which uses a UK validated set to estimate utilities from EQ-5D data. Doble and Lorgelly (2015) assessed the external validity of 10 mapping algorithms that mapped from EORTC QLQ-C30 onto the EQ-5D using data from the prospective longitudinal study Cancer 2015. This study included 1,834 patients, with a range cancer tumour sites, excluding leukaemia, and a range of disease stages including both local and metastatic disease. They reported that the algorithm created by Longworth et al. (2014) was one of the most computationally heavy but also performed well on a number of criteria, including extreme health states and having no statistically significant difference between observed and predicted QALYs over time.

By estimating the baseline EQ-5D utilities of the PORTEC2 trial (0.837 general population, 0.819 external beam radiation therapy [EBRT] receiving patients, 0.783 vaginal brachytherapy [VBT] receiving patients) and averaging the utility across the two treatments to give a utility estimate for the endometrial cancer population, the disutility for endometrial cancer is estimated to be −0.036. As the PORTEC2 trial indicates that QoL improves over time for endometrial cancer patients, reverting to (or exceeding) the QoL of the general population, this disutility is applied only for the first year following an endometrial cancer diagnosis.

5.1.4.2.3 Prophylactic hysterectomy

No studies were identified that could inform the disutility of prophylactic hysterectomy. In the base case, we assume no disutility from prophylactic hysterectomy, to reflect our belief that quality of life would be similar or better to the long term quality of life for endometrial cancer patients who have received hysterectomy and recovered from cancer. In the PORTEC2 trial this was at least as good as the general population utility and therefore results in a utility decrement of 0. In sensitivity analysis we set the disutility equal to the utility decrement from

endometrial cancer, under the assumption that prophylactic hysterectomy should not have a higher disutility than endometrial cancer.

5.1.4.2.4 Psychological impacts of Lynch syndrome testing and management on quality of life

No additional literature was identified following Snowsill et al. (2014) to estimate the psychological impact of Lynch syndrome testing on individuals offered testing for Lynch syndrome. As reported in Snowsill et al. (2014):

> Although diagnosis of LS can lead to interventions to reduce the chance of developing colorectal, gynaecological and other cancers, it can also lead to anxiety about developing these cancers and the need to make difficult decisions about whether or not to undergo risk-reducing surgeries. Furthermore, those diagnosed with LS must decide whether or not and how to inform relatives about their test results so that these relatives can consider whether or not they wish to be tested themselves. Given that anxiety is one aspect of HRQoL, such effects should be considered in the estimation of health-state utilities of probands and relatives.
>
> We identified just a single study of the cost-effectiveness of strategies for testing for LS [Wang and colleagues (2012)[79]] that incorporates disutilities associated with the psychological impact of testing. In this study it was assumed that such disutilities are transient, lasting 1 year in the base-case analysis.
>
> […]
>
> Disutilities due to testing itself and the test results were taken from the empirical study by Kuppermann and colleagues (2013).
>
> *—Page 162 of Snowsill et al. 2014[4]*

Snowsill et al. (2014) calculated disutility associated with testing for relatives using the utilities reported by Kuppermann et al. (2013)[157] in the following way:

> [We] assume that relatives who decline testing incur a disutility over 4 months of 0.04, equal to the utility of 0.76 (siblings who undergo testing, and test negative) minus 0.72 (siblings who decline testing). This disutility reflects anxiety the relative may feel in not knowing whether or not he or she has LS, with the corresponding substantial risk of developing cancer. Next, we assume a disutility of 0.02 for male relatives who are diagnosed with LS, equal to 0.76 for siblings who undergo testing and test negative, minus 0.74 for males who are tested positive for LS. Similarly, we assume a disutility of 0.06 for women who test positive and undergo TAHBSO, equal to 0.76 minus 0.70, and a disutility of 0.09 for women who test positive and decline TAHBSO, equal to 0.76 minus 0.67. The disutility is greater for women who decline TAHBSO presumably because they know that there remains a chance that they will develop gynaecological cancers. For women who test positive but are not offered TAHBSO as they are not at the appropriate age, we assume that the disutility of testing positive will be the same as for men who test positive, i.e. 0.02.
>
> *—Page 163 of Snowsill et al. 2014[4]*

Similarly, for probands:

> Kuppermann and colleagues did not measure the utility for probands who accepted testing and were diagnosed as LS negative. In the absence of this information, we assume that these individuals have no associated disutility due to genetic testing [...] Next, we estimate the disutility for probands of declining testing as 0.04, equal to the corresponding value for relatives […] Kuppermann and colleagues do not measure the utility for probands who accepted testing and were diagnosed with LS but were not offered any risk-reducing surgery, so we assume that the disutility for testing positive for male probands is the same as the disutility for male relatives, i.e. 0.02. For female probands who test positive and are not offered any risk-reducing surgery, we again assume the same disutility as for males, i.e. 0.02. For female probands who test positive and are offered prophylactic TAHBSO, we assume disutilities of 0.03 for those accepting surgery and 0.09 for those declining it. These disutilities are estimated by subtracting the utilities of 0.67 and 0.61 reported in Kuppermann and colleagues from the imagined utility of probands testing negative, which we estimate as the utility of probands declining testing (0.66) plus a utility of 0.04 for not declining testing taken from the relatives, to give a utility for probands testing negative for LS of 0.70. If a proband or relative declines testing but is still diagnosed with LS (by FH for probands or on account of being a FDR of a known carrier for relatives) and offered TAHBSO, we assume a disutility of 0.01 for probands and 0.04 for relatives (i.e. the same disutility as for probands or relatives testing positive), with an additional disutility of 0.06 for probands declining TAHBSO and 0.03 for relatives declining TAHBSO. For example, the total disutility for a proband declining testing and accepting TAHBSO would be 0.04 (declined testing) + 0.01 (offered TAHBSO) = 0.05, while the total disutility for a relative declining testing and declining TAHBSO would be 0.04 (declined testing) + 0.04 (offered TAHBSO) + 0.03 (declined TAHBSO) = 0.11.
>
> *—Page 164 of Snowsill et al. 2014*[4]

Table 55: PenTAG base case disutilities resulting from genetic testing

Result of genetic testing	Disutility	
	Males	**Females**
Proband		
Test declined, risk-reduction not offered	0.04	0.04
Test declined, accept risk-reduction	N/A	0.05
Test declined, decline risk-reduction	N/A	0.11
Test accepted, LS negative	0	0
Test accepted, LS positive, risk-reduction not offered	0.02	0.02
Test accepted, LS positive, accept risk-reduction	N/A	0.03
Test accepted, LS positive, decline risk-reduction	N/A	0.09
Relative		
Test declined, risk-reduction not offered	0.04	0.04
Test declined, accept risk-reduction	N/A	0.08
Test declined, decline risk-reduction	N/A	0.11
Test accepted, LS negative	0	0
Test accepted, LS positive, risk-reduction not offered	0.02	0.02
Test accepted, LS positive, accept risk-reduction	N/A	0.06
Test accepted, LS positive, decline risk-reduction	N/A	0.09

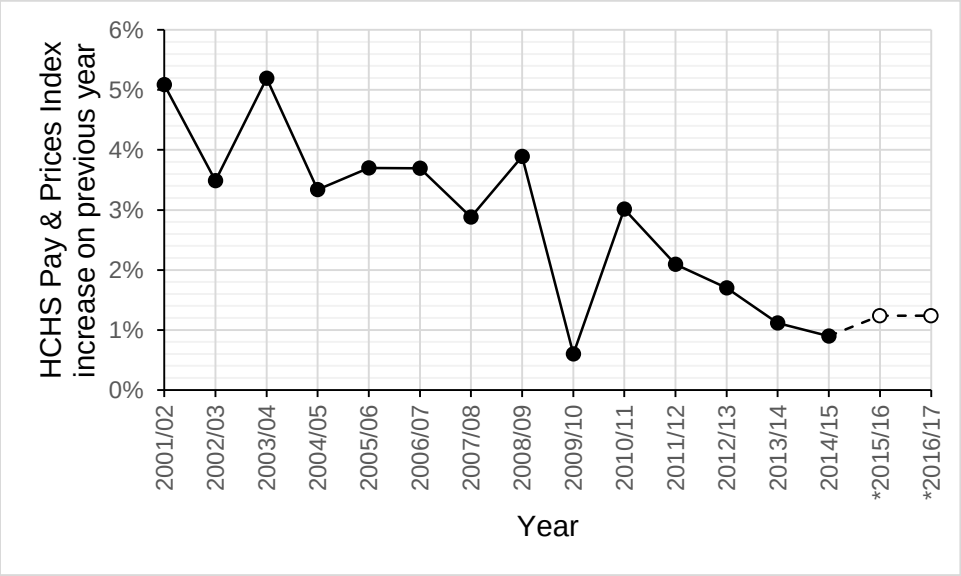
Key: LS, Lynch syndrome
Source: Snowsill et al. (2014)[4]

5.1.4.3 Resources and costs

5.1.4.3.1 Adjustments to 2016/17 prices

Costs were inflated to 2014/15 prices using the Hospital and Community Health Services (HCHS) Pay and Prices Index, and then to 2016/17 according to the average inflation in the three most recent recorded years (*Figure 33*).[158]

Figure 33: Historical and projected inflation of HCHS Pay & Prices Index



Key: HCHS, Hospital and Community Health Services
Notes: * Projection

5.1.4.3.2 Resource use

Colorectal surveillance

As described above (*Colorectal surveillance*, *page 176*), the model assumes biennial colonoscopy for individuals diagnosed with Lynch syndrome. The majority of those diagnosed with Lynch syndrome take up surveillance: 97% of those with a confirmed pathogenic mutation and 70% of those in whom Lynch syndrome is suspected without a causative mutation identified (see *Colonoscopy*, *page 183*).

Although acceptance is high, it is also recognised that implementation or concordance with the surveillance regimen is imperfect, for a number of reasons (e.g., service delivery, patient circumstances and factors). It was estimated that 12.7% of planned colonoscopies would be missed, based on a Regional Familial Colorectal Cancer Registry study by Newton et al.[159] This parameter is not assumed to affect the effectiveness of colonoscopy, and therefore its impact on cost-effectiveness is not as expected – increasing this parameter in a sensitivity analysis would reduce the costs of surveillance without reducing effectiveness, and would therefore improve cost-effectiveness of diagnostic strategies. For this reason, this parameter is not subjected to sensitivity analyses.

It is important (especially for individuals with Lynch syndrome who are more susceptible to proximal colon cancer than the general population) that colonoscopy is complete (including intubation of the caecum). For this reason, it is recommended that repeat colonoscopies are conducted when colonoscopy is incomplete. The probability of any colonoscopy being incomplete and needing repeating was estimated as 7.7%, based on a national colonoscopy audit.[160] It was assumed at most one repeat colonoscopy would be performed.

It was assumed that surveillance colonoscopies for Lynch syndrome would start at age 25 and end at age 75, based on BSG/ACPGBI guidelines.[43]

Complications

Based on a national colonoscopy audit[160] it was assumed that for each 100,000 colonoscopies, there would be 260 bleeding events and 40 perforation events. It was also assumed there would be 8.3 deaths per 100,000 colonoscopies.[43]

Bleeding events were only modelled if they resulted in admission, and it was estimated that 21% of bleeds would result in admission.[160] Of these, approximately 18% would be "moderate" bleeds and 9% would be "severe" bleeds.[160]

Aspirin

As described in *Section 5.1.4.1.4 (page 190)*, 80.4% of individuals with Lynch syndrome are offered aspirin chemoprevention and accept it. Of these, 59.0% are concordant with the protocol and receive aspirin for four years, while the remaining individuals are assumed to discontinue immediately.

The daily dose modelled is 600 mg.

Colorectal cancer

Diagnosis

All colorectal cancer patients are assumed to incur the cost of diagnosis once, in the year of diagnosis.

Primary chemotherapy and radiotherapy

Rectal cancer

For rectal cancer patients, 79% are estimated to be operable, and of these 42.5% are estimated to present as emergency cases, with the remaining 57.5% scheduling elective surgery.[116] Of the emergency cases, 11% are estimated to receive postoperative chemoradiotherapy (for two weeks). Of the elective cases, 82% are predicted by MRI to have clear margins after resection, and 60% of these have preoperative chemoradiotherapy. A further 4.4% have postoperative chemoradiotherapy (11% of the 40% not receiving preoperative chemoradiotherapy). All patients not predicted to have clear margins after resection receive a course of chemoradiotherapy, and 88% of these go on to have surgery.[116]

Post-surgery chemotherapy courses are given to 28% of patients with Dukes' B rectal cancer and 75% of patients with Dukes' C rectal cancer. Chemotherapy is not given to Dukes' A patients, and Dukes' D patients receive palliative therapy, which is detailed in *Palliative care* (*page 210*).

Colon cancer

Adjuvant chemotherapy is given to 0% of Dukes' A, 39% of Dukes' B and 89% of Dukes' C colon cancer patients.[116]

Dukes' D colon cancer patients may receive downstaging chemotherapy for liver metastases, but this is costed under recurrence chemotherapy and surgery.

Primary surgery

All patients diagnosed with colorectal cancer are assumed to receive primary surgery, as described in *Section 5.1.2.2.7*, *page 176*.

Surveillance

In the first five years after diagnosis, colorectal cancer patients have a surveillance pathway which includes CEA tests, CT scans, colonoscopies and clinical consultations. Patients are assumed to receive 3-monthly CEA tests for the first two years, then 6-monthly tests for the following three years, plus CT scans at 12 and 24 months and annual clinical consultation. Patients are also assumed to receive a colonoscopy at 12 months and then every five years thereafter (unless they already receive Lynch syndrome surveillance colonoscopies). *Table 56* shows this pathway. As in Trueman et al.[116] a weighted average cost per year is calculated.

Table 56: Surveillance pathway resource use

Year	CEA test	CT scan	Clinical consultation
Year 1	4	0	1
Year 2	4	1	1
Year 3	2	1	1
Year 4	2	0	1
Year 5	2	0	1

Surgery and chemotherapy for recurrence

All patients dying from colorectal cancer within five years of diagnosis incur the cost of surgery and chemotherapy for recurrence in the year of their death.

Stoma care

All patients with colorectal cancer incur average stoma care costs (i.e., the model does not actually track whether a patient has a stoma or not). It was estimated from Trueman et al.[116] that 67% of rectal cancer patients would require a stoma after surgery compared to 14.5% of colon cancer patients. Of these, 26.6% would be reversed and the patient would not require long term stoma care. On this basis it was estimated that 11% of colon cancer patients and 49% of rectal cancer patients would require a permanent stoma.

Palliative care

All patients dying from colorectal cancer (at any time after diagnosis) incur the cost of palliative care in the year of their death.

Gynaecological cancer risk reduction

Women diagnosed with Lynch syndrome may be offered interventions to reduce their risk of developing gynaecological cancers (in the model only endometrial cancer is included).

Women with Lynch syndrome were assumed to be offered gynaecological surveillance at age 35 (in line with ESMO guidelines[118]) or at the time of their diagnosis (whichever is later).

Initial risk reduction

As described above (*Gynaecological state on entry*, *page 166*), the probability that a woman diagnosed with Lynch syndrome opts for surveillance, prophylactic surgery, or no intervention, at the time of diagnosis was estimated based on the results of the Northern Genetics Service audit (*Table 57*), by dividing the number of women opting for each intervention in each age range (except ≤ 35) by the total number of women, excluding those with previous cancer or whose results were not recorded. The resulting probabilities of opting for surveillance or prophylactic surgery at the time of diagnosis are given in *Table 58*.

Table 57: Risk-reducing measures of women with Lynch syndrome mutations

Age group	Discussed only	Surveillance	Prophylactic surgery	Previous cancer	Not recorded
≤ 35	10	6	0	0	1
36–45	3	9	3	0	1
46–60	4	11	9	6	2
> 60	0	2	12	4	4

Source: Northern Genetics Service audit (Lorraine Cowley, Principal Genetic Counsellor; personal communication, 20th November 2012)

Table 58: Initial gynaecological cancer risk reduction

Age at diagnosis (years)	Surveillance	Prophylactic H-BSO
< 35	0.0%	0.0%
35–44	60.0%	20.0%
45–59	45.8%	37.5%
60–69	14.3%	85.7%
70+	0.0%	85.7%

Key: H-BSO, hysterectomy and bilateral salpingo-oophorectomy

Subsequent risk reduction

Women diagnosed with Lynch syndrome can change their risk reduction subsequently. It was assumed that all women still receiving surveillance would stop receiving surveillance at age 70. It was assumed that at age 35 women would be offered surveillance or prophylactic H-BSO. It was assumed that at age 45 and at age 60 women would be offered prophylactic H-BSO. The probability of receiving prophylactic H-BSO at age 45 was estimated as 21.9% as 21.9% of the 80% not already with prophylactic H-BSO is 17.5%, which is the increase in H-BSO between the 35–44 and 45–59 age groups. Similarly it was estimated that 77.1% of women would receive prophylactic H-BSO at age 60 (see *Table 59*).

Table 59: Subsequent gynaecological cancer risk reduction

Reaching age (years)	Surveillance	Prophylactic H-BSO
35	60.0%	20.0%
45	0.0%	21.9%
60	0.0%	77.1%

Key: H-BSO, hysterectomy and bilateral salpingo-oophorectomy

Endometrial cancer

As previously,[4] endometrial cancer treatment was assumed to consist of surgery for all patients and adjuvant radiotherapy and/or chemotherapy for some patients.

Surgery

All women diagnosed with endometrial cancer are assumed to receive surgery.

Radiotherapy

Radiotherapy is assumed to be used in 33% of Stage I patients, 100% of Stage II/III patients and 0% of Stage IV patients.[161] It is estimated that this results in radiotherapy being used in 47% of patients overall.

Adjuvant chemotherapy

Chemotherapy is assumed to be used in 0% of Stage I patients, 50% of Stage II/III patients and 100% of Stage IV patients.[161] It is estimated that this results in chemotherapy being used in 18% of patients overall.

We model a combination regimen of carboplatin and paclitaxel (*Table 60*). This has been noted as a popular and reasonable regimen in the British Gynaecological Cancer Society draft guidelines,[162] which also state there is currently no evidence to conclude any adjuvant chemotherapy regimen is superior to another.

Table 60: Chemotherapy regimen for endometrial cancer

Day	Drug	Dose	Administration
1	Paclitaxel	175 mg/m²	IV over 3 hours
1	Carboplatin	AUC 5–6 mg · min/mL	IV over 60 minutes

Key: AUC, area under the curve; IV, intravenous

The required dose to obtain a specific carboplatin AUC is given by the Calvert formula[163]:

$$\text{Dose (mg)} = \text{Target AUC (mg·min/mL)} \times (\text{GFR (mL/min)} + 25)$$

We assumed an average GFR of 82.7 mL/min, based on a study of 1,218 patients in a Belgian study.[164] We therefore obtained a dose of 592.35 mg carboplatin per cycle (assuming a target AUC of 5.5 mg · min/mL).

We assume an average body surface area of 1.71 m², based on the average body surface area for women with cancer as reported by Sacco et al. 2010,[165] which leads to a dose of 299 mg paclitaxel.

5.1.4.3.3 Unit costs

Diagnostic tests

Costs of tumour testing

Costs of the preliminary tumour tests have been obtained directly from laboratories in the UK (see *Table 61*). Where possible, these have been sourced from multiple genetics services via the UK Genetics Testing Network (UKGTN) to produce a cost representative across the

UK. Unit costs from personal communications received during the completion of this report and Snowsill et al. (2014)[4] have been inflated to 2016.

Costs of genetic testing

Costs for genetic tests (for probands and relatives) are taken directly from genetic testing laboratories, as reported via the UKGTN.[166] As in Snowsill et al. (2014), only costs applicable to the NHS are collected (private fees are excluded where possible) and overall values for each cost are calculated across the laboratories that supply each given test. Available genetic tests are individual sequencing tests for probands for *MLH1*, *MSH2*, *MSH6* and *PMS2*; individual targeted tests for *MLH1*, *MSH2*, *MSH6* and *PMS2* for relatives; a combined *MLH1*, *MSH2* sequencing test for probands; a combined *MSH2*, *MSH6* sequencing test for probands; a combined *MLH1*, *MSH2* and *MSH6* sequencing test for probands; and, at one centre, a combined sequencing test for all four genes. As the PenTAG model assumes probands will receive testing for four genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*), the total cost for testing all four genes is based on the average of the plausible combinations of tests that can be found in current practice. This cost does not include the added costs of having to use multiple centres to produce the results. There is some indication that labs are increasingly testing MMR genes as part of NGS multi-gene panels (based on clinical opinion from IMF, 2016). The result of this is both to reduce costs and increase the yield of conditions causative of CRC. Costs are therefore explored in univariate sensitivity analyses.

As in Snowsill et al. (2014), genetic counselling is assumed to occur after initial tumour testing and before genetic testing. Genetic counselling remains a parameter without a standard unit cost. As such, it was calculated using the same approach as detailed in Snowsill et al. (2014), with updated costs. To calculate the time and staff involved in genetic counselling Snowsill et al. corresponded with Professor Mary Porteous of the South East Scotland Genetic Service, based at the Western General Hospital in Edinburgh (2013, personal communication). As detailed in Snowsill et al. (2014):

> In this centre genetic counselling occurred, where applicable, after the tumour tests (IHC and/or MSI) for the proband. Generally, probands received a maximum of a single 45-minute session with a band 7 counsellor before gene testing, and a 30-minute session to discuss the results. The same was also true of the relatives, though in practice the total 75 minutes could be split in various ways (for example, sometimes relatives would have a group session then return for a shorter individual session before they were tested). In this centre the cost of genetic counselling incurred for a relative of a proband was therefore the same as that for the proband.
>
> *—Page 168 of Snowsill et al. 2014[4]*

The cost per hour of the band 7 counsellor (£50) was taken from the Health and Social Care Unit Costs (2014)[167] and uprated to 2016 costs, as no equivalent cost was available from the most recent edition. The cost per hour of a band 5 hospital nurse (£43) was taken from Health and Social Care Unit Costs (2015) and uprated to 2016 costs.[158]

As different genetic centres have different approaches to genetic counselling, sensitivity analyses are performed on the cost of counselling, halving and doubling the time spent on each individual.

Table 61: Costs of diagnostic tests and genetic counselling

Test	Patient	Base case cost	Base case source
MSI	Proband	£202	Average from UKGTN 2016 (reported for Newcastle, Oxford, Birmingham and Sheffield)
IHC	Proband	£210	Average of UKGTN 2016 (reported for University College London only), Dr Mark Arends (Department of Pathology, University of Cambridge) and Dr Ian Frayling (All-Wales Genetics Service) (£220-£240) 2012 costs updated to 2015/2016 costs
BRAF V600E	Proband	£119	Average of • £140 personal communication with Mr Michael Gandy (UCL-Advanced Diagnostics) • £117 East of Scotland Regional Genetic Service[168] • £85 All Wales Molecular Genetics Laboratory[169] 2012 costs • £65 North West Regional Genetics Service (UKGTN 2016) (all updated to 2015/2016 costs)
MLH1 promoter methylation testing	Proband	£136	UKGTN 2016 (reported for Newcastle, Cardiff and London)
Proband genetic test, all four genes	Proband	£1,276	UKGTN 2016 (weighted average of available test)
Proband genetic counselling	Proband	£64	The PSSRU (2014, 2015) and personal communication with Professor Mary Porteous (SE Scotland Genetic Service) from 2013
Targeted genetic test for relatives (*MLH1*)	Relative	£166	UKGTN 2016
Targeted genetic test for relatives (*MSH2*)	Relative	£161	UKGTN 2016
Targeted genetic test for relatives (*MSH6*)	Relative	£161	UKGTN 2016
Targeted genetic test for relatives (*PMS2*)	Relative	£165	UKGTN 2016
Relative genetic counselling	Relative	£64	The PSSRU (2014,2015)[158, 167] and personal communication with Professor Mary Porteous (SE Scotland Genetic Service) from 2013

Sources: PSSRU (2014, 2015),[158, 167] UKGTN 2016[166]

Colorectal surveillance

The unit costs of surveillance colonoscopies for individuals with Lynch syndrome were estimated from the NHS reference costs 2014–15[170] and uprated to 2016–17 prices as described in *Section 5.1.4.3.1*. The Healthcare Resource Groups (HRGs) FZ51Z, FZ52Z and FZ53Z were used as previously[4] giving a unit cost of £585.80 per colonoscopy (see *Table 62*).

Table 62: Reference costs for colonoscopies

HRG	Description	Number of colonoscopies	Unit cost (£)	Total cost (£)
FZ51Z	Diagnostic Colonoscopy, 19 years and over	162,933	519.42	84,630,117
FZ52Z	Diagnostic Colonoscopy with Biopsy, 19 years and over	153,795	604.02	92,894,549
FZ53Z	Therapeutic Colonoscopy, 19 years and over	116,071	601.86	69,858,823
Weighted average (2014–15 prices)		432,799	571.59	247,383,489
Weighted average (2016–17 prices)			585.80	

Key: HRG, Healthcare Resource Group
Source: NHS reference costs 2014–15[170]

Complications

The unit costs of complications from colonoscopies were estimated from NHS reference costs (*Table 63*).[170]

Table 63: Unit costs of complications from colonoscopies

Complication	NHS reference cost HRG	Unit cost	
Price year		*2014/15*	*2016/17*
Bleed requiring admission			
• Mild	FZ38P Gastrointestinal Bleed without Interventions, with CC Score 0-4[a]	£462	£473
• Moderate	FZ38J–FZ38L Gastrointestinal Bleed with Single Intervention[a]	£1,110	£1,138
• Severe	FZ38G–FZ38H Gastrointestinal Bleed with Multiple Interventions[b]	£4,287	£4,394
Perforation	FZ77C–FZ77E Major Large Intestine Procedures, 19 years and over[b]	£4,790	£4,909
Death	Assumed same as perforation	£4,790	£4,909

Notes: [a] Non-elective short stay only; [b] Non-elective long stay and non-elective short stay
Key: CC, complications and comorbidities; HRG, healthcare resource group

Colorectal cancer

As previously,[4] many of the costs associated with colorectal cancer in the model are based on the work of Trueman et al. (2007).[116] The main exception to this is the cost of primary surgery (see *Primary surgery*, *page 208*).

Diagnosis

The cost of diagnosis is incurred at the time of CRC diagnosis and is estimated to be £1,022 in 2016/17 prices (inflated from £790 in 2004/05 prices).[116]

In addition, it is assumed that patients with Stage II (Dukes' B) colon cancer will receive MSI testing to predict their response to 5-FU-based chemotherapy, at a cost of £202 (see *Costs of tumour testing*, *page 204*).

Primary chemotherapy and radiotherapy

Trueman et al. model pre/postoperative chemoradiotherapy and adjuvant chemotherapy.[116]

Rectal cancer

In Trueman et al. the cost of pre/postoperative chemoradiotherapy was £2,263 (2004/05 prices) and the cost for a full chemotherapy course after surgery was £11,209 (2004/05 prices).

When weighted by the resource use, the relevant cost according to rectal cancer stage are shown in *Table 64*.

Table 64: Cost of chemotherapy and radiotherapy for rectal cancer patients

Rectal cancer stage	Pre/postoperative chemoradiotherapy		Chemotherapy		Total
	%	Subtotal	%	Subtotal	
Dukes' A	35.9%	£1,049.30	0%	£0.00	£1,049.30
Dukes' B	35.9%	£1,049.30	21.8%	£3,156.37	£4,205.67
Dukes' C	35.9%	£1,049.30	58.3%	£8,454.57	£9,503.87

Colon cancer

The cost of chemotherapy for colon cancer was £11,209 in 2004/05 prices in Trueman et al.,[116] corresponding to a cost of £14,494 in 2016/17 prices.

Primary surgery

As previously,[4] the costs of colorectal cancer surgery were estimated according to the type of surgery performed and whether the patient had Lynch syndrome or not (since proximal colorectal cancers are more common in individuals with Lynch syndrome). Stoma reversal costs were included as previously.[4] Unit costs were updated using the NHS reference costs 2014–15[170] and uprated to 2016–17 prices.

Table 65: Unit costs for colorectal cancer surgical procedures

Surgery	HRG	Unit cost (£2014–15)	Unit cost (£2016–17)
Segmental resection (proximal without exteriorisation)	FZ75 Proximal Colon Procedures, 19 years and over	6,286.10	6,442.38
Segmental resection (distal without exteriorisation)	FZ76 Distal Colon Procedures, 19 years and over	5,920.68	6,067.87
Segmental resection (with exteriorisation)	FZ74 Complex Large Intestine Procedures, 19 years and over	7,671.24	7,861.95
Subtotal colectomy with ileorectal anastomosis			
Anterior resection			
Proctocolectomy with ileal pouch anal anastomosis			
Stoma reversal	FZ50 Intermediate Large Intestine Procedures, 19 years and over	420.77	431.23

Key: HRG, Healthcare Resource Group
Source: NHS reference costs 2014–15[170]

Table 66: Estimated costs of surgery for CRC

Surgical extent	Unit cost (£)
Segmental resection	
General population	6,500.58
Lynch syndrome	6,604.77
Subtotal colectomy with ileorectal anastomosis	7,878.59
Rectal excision	7,938.81
Proctocolectomy	7,976.66

Note: 2016–17 prices

Surveillance

We estimate that CEA tests cost £13.64 each (by inflating the cost of £10.55 from Trueman et al.[116]). CT scans are assumed to be three areas with contrast, at a cost of £127.63 (inflated from £124.53 in 2014–15 NHS reference costs[170]). Clinical consultations are estimated to cost £128.17 each, inflated from £125.06 in 2014–15 NHS reference costs (consultant-led, service code 104 colorectal surgery).

Trueman et al. estimated five years of surveillance costs for colorectal cancer, from which we derive an average cost per person-year of £232 for rectal cancer and £229 for colon cancer, as shown in *Table 67*.

Table 67: Derivation of colorectal cancer surveillance costs (excluding colonoscopy)

Year	Rectal cancer patients	Colon cancer patients	Unit cost (2016/17 prices)
1	7,029	17,209	£182.74
2	4,570	11,142	£310.36
3	2,533	7,008	£283.08
4	1,316	4,356	£155.45
5	571	2,337	£155.45
Total person-years	16,019	42,052	
Total cost	£3,713,208	£9,627,079	
Average cost per person-year	£231.80	£228.93	

The model does not distinguish between colon and rectal cancer after the year of diagnosis, so these costs are applied as an average, weighted according to whether the patient has Lynch syndrome or not. Corresponding costs of £229 and £230 are used for patients with and without Lynch syndrome respectively.

In addition to this the model includes colonoscopy every five years starting at 12 months, at a cost of £586 (see *Colorectal surveillance*, *page 207*).

Surgery and chemotherapy for recurrence

A single cost of £11,999 was applied if a patient died within five years from diagnosis of rectal cancer, and similarly £12,354 in the case of colon cancer. These are inflated from estimates of £9,279 and £9,554 from Trueman et al. in 2004/05 prices.[116]

As above, since the model does not track the site of cancer after the year of diagnosis, these are applied as weighted averages of £12,333 and £12,236 for patients with and without Lynch syndrome respectively.

Stoma care

The annual cost of stoma care was estimated as £1,279 (2004/05 prices) by Trueman et al.[116] and this was inflated to £1,654 in 2016/17 prices.

Based on 11% of colon cancer and 49% of rectal cancer patients requiring a permanent stoma, annual average costs of stoma care of £214 and £388 were applied to patients with or without Lynch syndrome respectively.

Palliative care

Trueman et al. estimated costs of £7,703 and £7,016 for palliative care for colon and rectal cancer patients respectively.[116] These were inflated to £9,961 and £9,072 in 2016/17 prices and then used to estimate costs of £9,907 and £9,665 for patients with and without Lynch syndrome respectively.

Gynaecological surveillance

Gynaecological surveillance was assumed to include annual CA125 testing, gynaecological examination, transvaginal ultrasound and endometrial aspiration biopsy. The total annual cost was estimated to be £473.41.

CA125 testing

The cost of CA125 testing was estimated to be £21.71, based on an approximate cost of £20 given in an NHS news story in 2011.[171]

Gynaecological examination

The cost of a gynaecological examination was estimated to be £122.93. This was based on the NHS reference cost WF01A for consultant-led, non-admitted face-to-face attendance (follow-up) in the gynaecology service (service code 502), £119.95 in 2014/15 prices.[170]

Transvaginal ultrasound

The cost of transvaginal ultrasound was estimated to be £160.65. This was based on the NHS reference cost MA36Z "Transvaginal ultrasound" (£156.75 in 2014/15 prices).

Endometrial aspiration biopsy

The cost of endometrial aspiration biopsy was estimated to be £168.12. This was based on the NHS reference cost MA25Z "Minimal upper genital tract procedures" (£164.04 in 2014/15 prices).

Prophylactic gynaecological surgery

The cost of prophylactic gynaecological surgery (hysterectomy and bilateral salpingo-oophorectomy) was estimated to be £3,428. This was based on an average cost of £3,345 for MA07E–MA07G "Major open upper genital tract procedures" and MA08A–MA08B "Major, laparoscopic or endoscopic, upper genital tract procedures" from NHS reference costs.[170]

Endometrial cancer

Surgery

The cost of surgical management of endometrial cancer was estimated to be £4,005. This was based on an average cost of £3,907 for MA06A–MA06C "Major, open or laparoscopic, upper or lower genital tract procedures for malignancy" from NHS reference costs.[170]

Radiotherapy

Havrilesky et al.[161] estimated a cost of $7,895 (US dollars) for a course of radiotherapy for endometrial cancer. It was estimated that this corresponded to a cost of £5,870 in 2016/17 prices (based on the same methodology as previously employed[4]).

Adjuvant chemotherapy

The eMit database[172] was used to estimate the average cost of carboplatin (4.41p per mg) and paclitaxel (8.09p per mg) reflecting average acquisition costs (weighted across pack sizes by total number of mg reported).

The cost of administering the chemotherapy regimen was estimated to be £399.09 per cycle. This is based on a cost of £389.41 for SB14Z "Deliver complex chemotherapy, including prolonged infusional treatment, at first attendance".[170]

The total cost for a course of chemotherapy was estimated to be £1,797.63 per patient receiving chemotherapy.

Aspirin

It was assumed that aspirin would be prescribed by general practitioners and dispensed in the community, therefore the reference case unit costs are the list prices or the prices on the NHS drug tariff.

A pack of 100 enteric coated tablets of 300 mg aspirin costs £20.33 in the BNF[173] and £20.34 in the NHS drug tariff.[174] The cost of £20.33 was used, which corresponds to a daily cost (600 mg) of £0.41 and an annual cost of £148.51.

5.1.5 Quality assurance

The independent economic assessment was conducted by extending the model from Snowsill et al. 2014[4] which had already been quality assured. All parts of this model had been checked by at least one developer not responsible for developing that component, using code review and black box testing.

Extensions to the previous model were highlighted as requiring checking and were then checked by the developer not responsible for developing that extension. The checking involved code review and checking that input parameters matched the described sources. A parallel build was also conducted for one component. This revealed a small discrepancy in calculated results which was then resolved through discussion.

5.2 Cost effectiveness results

The population simulated in the model comprises probands (individuals diagnosed with colorectal cancer for whom different diagnostic strategies for Lynch syndrome may be employed) and relatives who would be identified if a Lynch syndrome causing mutation were diagnosed in the proband. Since the majority of probands do not have Lynch syndrome, likewise the majority of relatives also do not have Lynch syndrome.

Throughout this result all costs and QALYs are discounted at 3.5% per annum (unless otherwise stated), and life years are not discounted.

5.2.1 Base case results

5.2.1.1 Characteristics of the simulated population

In the base case, there are 238,175 simulated individuals (reflecting an annual cohort), of whom 34,025 (14.3%) are probands (i.e., individuals with a colorectal cancer diagnosis) and 204,150 (85.7%) are relatives of probands. Of the probands, 956 (2.8%) are expected to have Lynch syndrome, with a corresponding 2,524 (1.2%) relatives (see *Table 68*). Of the probands, 55.0% are men, while only 37.6% of relatives are men.

Table 68: Simulated population

Simulated individuals	With Lynch syndrome	Without Lynch syndrome	Total
Probands	956 (2.8%)	33,069 (97.2%)	34,025 (100%)
Relatives	2,524 (1.2%)	201,626 (98.8%)	204,150 (100%)
Total	3,480	234,695	238,175

The average age of probands at time of diagnosis was 72.7 years (without Lynch syndrome) and 58.0 years (with Lynch syndrome), reflecting the widely observed earlier age of colorectal cancer incidence in individuals with Lynch syndrome. The mean age at entry for relatives was 44.4 years without Lynch syndrome and 43.2 years with Lynch syndrome.

5.2.1.2 Cost-effectiveness results

Table 69 reports the summary cost-effectiveness results for the 10 strategies. We present both ICERs versus no testing (Strategy 1) plus the comparative ICERs for all strategies. We note that the optimal strategy (highest incremental net health benefit [INHB] at a willingness to pay threshold of £20,000 per QALY gained) is IHC testing followed by both BRAF and *MLH1* promoter methylation testing (Strategy 5). Universal genetic testing has the highest ICER versus no testing: £25,884 per QALY gained.

Table 69: Summary base case cost-effectiveness results

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,508,052	£743,298,306	—	—	—
2: IHC	3,510,017	£767,955,447	£12,553	731.5	£60,967
3: IHC plus *BRAF*	3,509,977	£765,532,726	£11,553	812.9	£37,495
4: IHC plus *MLH1* promoter methylation	3,509,965	£765,535,788	£11,672	793.3	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,509,937	£764,048,240	£11,005	848.0	£11,008
6: MSI	3,509,926	£769,249,096	£13,849	576.3	Dominated by 2
7: MSI plus *BRAF*	3,509,832	£763,660,095	£11,438	762.0	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,509,796	£763,503,459	£11,589	733.2	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,509,721	£761,784,044	£11,076	744.7	Extended dominated by 1 and 5
10: Universal genetic testing	3,509,987	£793,380,127	£25,884	−569.2	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Figure 34: Incremental discounted costs and QALYs for all probands and relatives

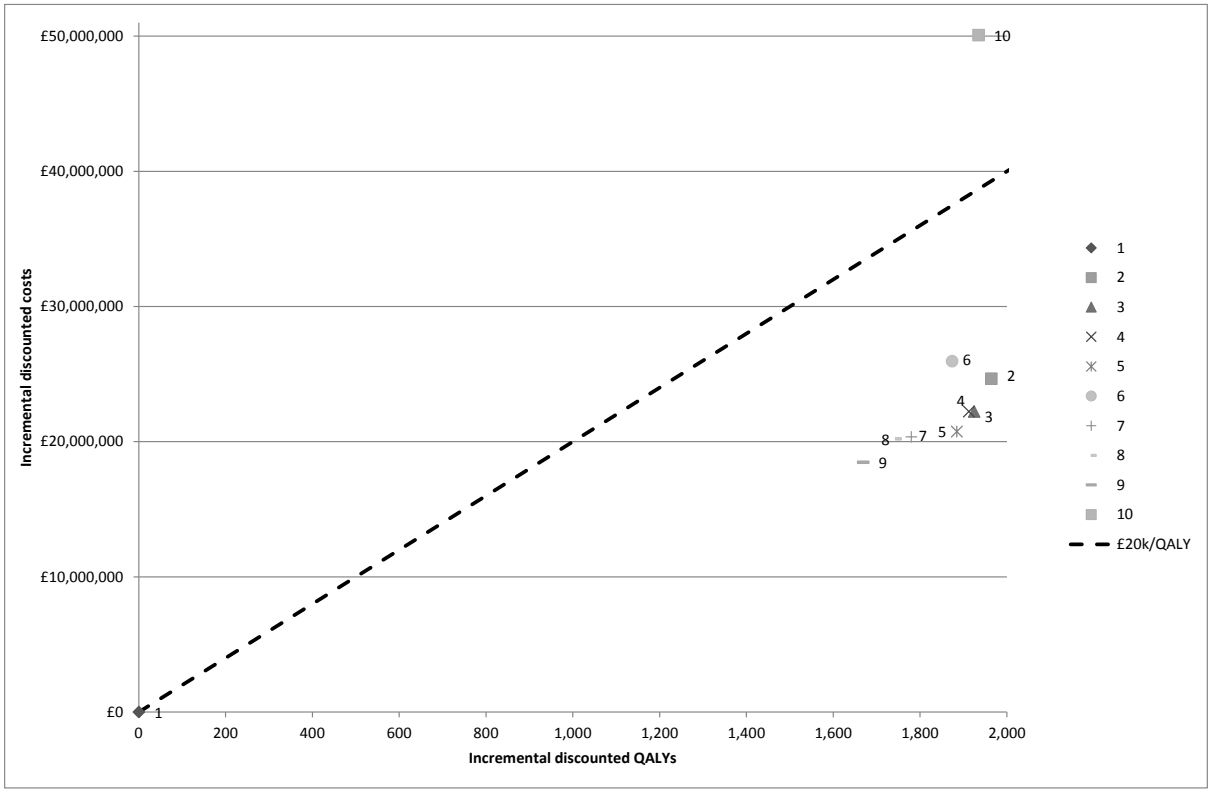


Figure 34 shows that all testing strategies are cost-effective at a willingness to pay threshold of £20,000 per QALY compared to the no testing strategy (Strategy 1), with the exception of universal genetic testing.

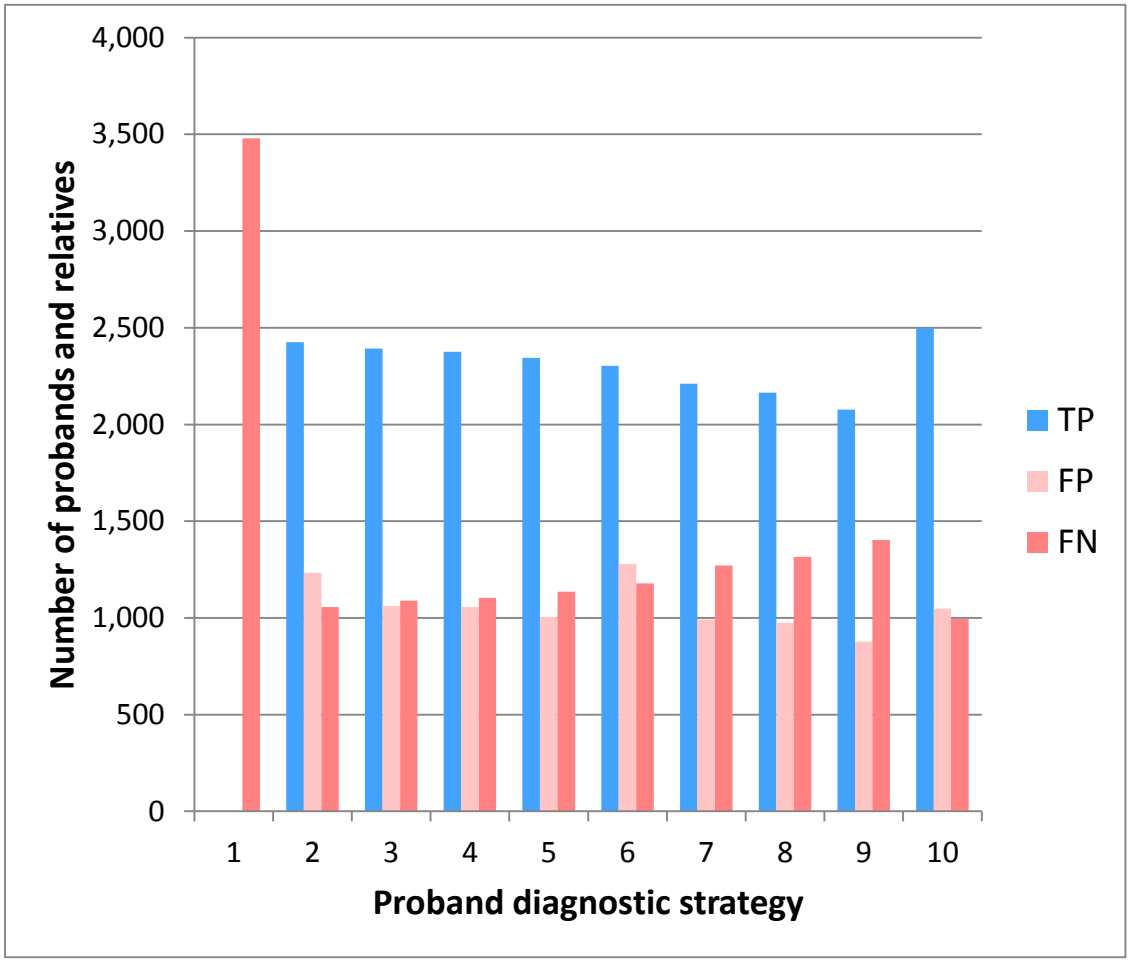
In a fully incremental analysis, four strategies are on the cost-effectiveness frontier: 1 (No testing), 2 (IHC), 3 (IHC plus *BRAF*) and 5 (IHC plus *BRAF* and *MLH1* promoter hypermethylation). The remaining strategies are dominated (i.e., more costly and less effective than one or more comparators) or extended dominated (i.e., more costly and less effective than a combination of other comparators).

We now examine the drivers of these cost effectiveness results by looking at the test accuracy, life expectancy and costs of each strategy.

5.2.1.3 Test accuracy results

Figure 35 shows the number of diagnoses made by each strategy. Of most interest is the number of incorrect diagnoses. Aside from Strategy 1, Strategy 9 (MSI followed by *BRAF* and *MLH1* methylation) has the highest number of false negatives (people with undiagnosed Lynch syndrome) and the least false positives (people diagnosed with Lynch syndrome who do not have it). This means Strategy 9 will likely have few unnecessary Lynch syndrome prevention costs, but more cancer treatment costs. Universal genetic testing has the least false negatives, which is likely to reduce cancer treatment costs. The highest number of false positives are found in Strategy 2 (IHC) and Strategy 6 (MSI), which is likely to lead to additional unnecessary surveillance costs. The only strategies where the false positive rate is higher the false negative rate is where only one test is used in a sequence. This makes sense as multiple tests in a sequence are used to enrich a population and reduce the number of false positives.

Figure 35: Number of probands and relatives identified by each strategy



Key: FN, false negative; FP, false positive; TP, true positive
Notes: True negatives have not been shown in the interest of clarity. The number of true negatives is substantially larger than the other three diagnoses.

The sensitivities and specificities of the different strategies are shown in *Table 70*. These outputs can be calculated for probands only or for the overall population (including probands and relatives). As can be seen, diagnostic performance follows a similar pattern in the two populations, but sensitivity and specificity are generally lower in the overall population. Performance is reduced through two mechanisms. Firstly, in the event of a positive diagnosis but without identification of a causative mutation, all first-degree relatives are treated as though they have Lynch syndrome and more distant relatives are treated as though they do not have Lynch syndrome. Secondly, some relatives opt not to receive predictive testing for Lynch syndrome.

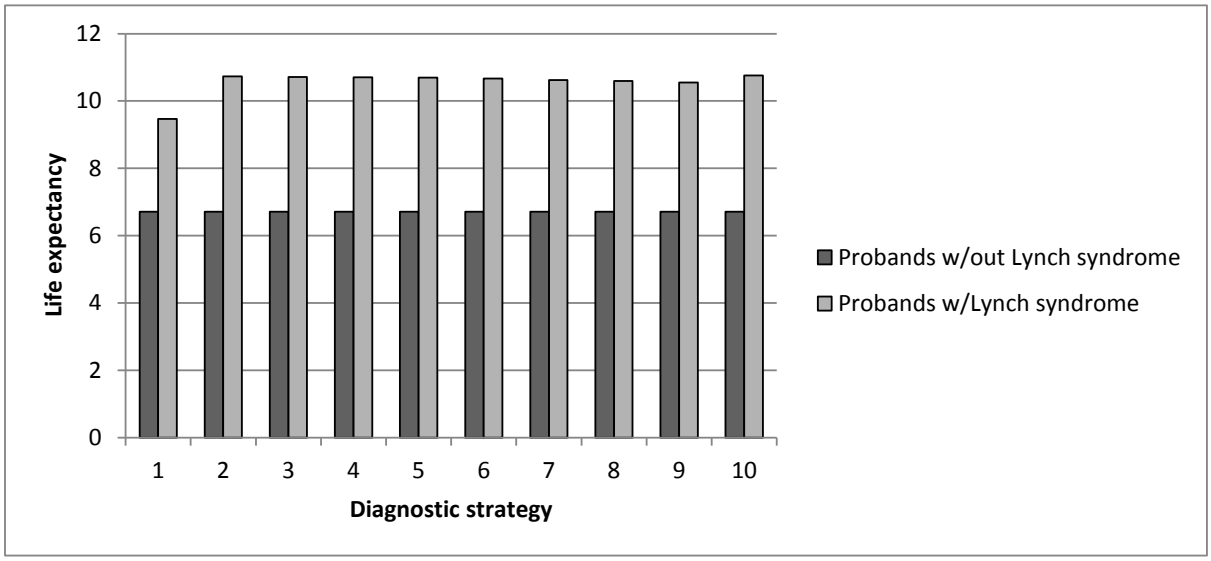
Table 70: Sensitivity and specificity for different strategies

Strategy	Probands only		Overall population	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
1: No test	0.00	100.00	0.00	100.00
2: IHC	70.35	99.80	69.67	99.47
3: IHC plus *BRAF*	69.40	99.94	68.73	99.55
4: IHC plus *MLH1* promoter methylation	68.93	99.93	68.26	99.55
5: IHC plus *BRAF* and *MLH1* promoter methylation	68.04	99.97	67.38	99.57
6: MSI	66.80	99.72	66.17	99.46
7: MSI plus *BRAF*	64.13	99.93	63.51	99.58
8: MSI plus *MLH1* promoter methylation	62.79	99.93	62.18	99.58
9: MSI plus *BRAF* and *MLH1* promoter methylation	60.28	99.98	59.69	99.63
10: Universal genetic testing	71.43	99.98	71.53	99.55

5.2.1.4 Long term clinical outcomes

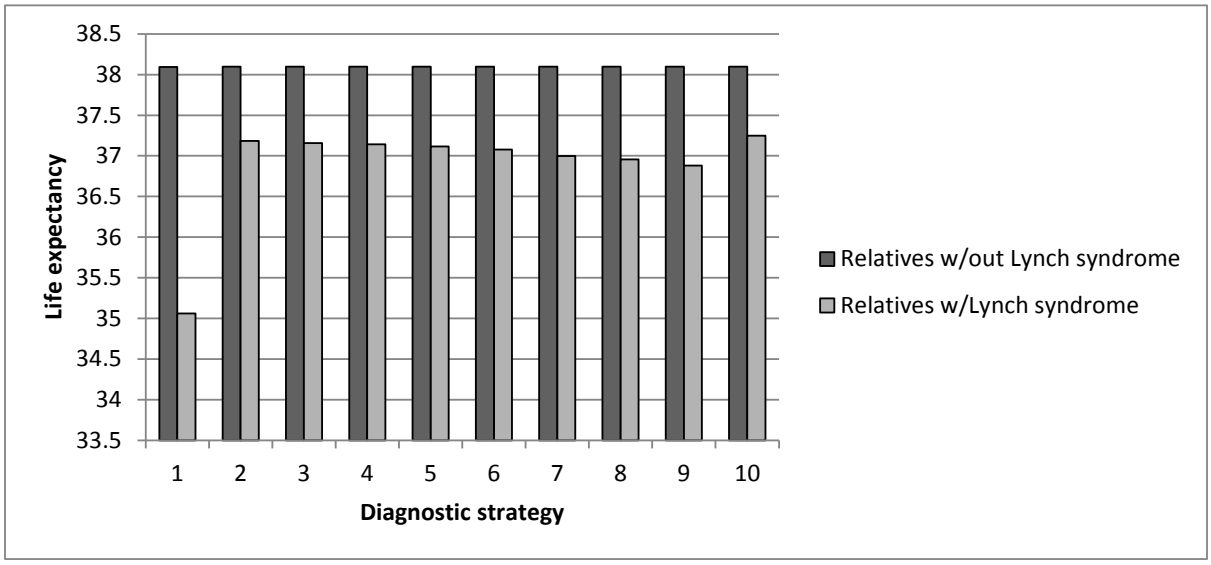
Figure 36 and *Figure 37* give the estimated life years for probands and relatives, according to their Lynch syndrome status. As expected, life expectancy is much longer in relatives (they are more likely to enter the model at a younger age and they are healthy at time of entry to the model). We find that testing for Lynch syndrome improves the life expectancy of both relatives and probands, a probable consequence of the reduction in CRC (index and metachronous) and endometrial cancer from the preventative measures offered to patients in who Lynch syndrome is diagnosed. For probands, Lynch syndrome probands consistently have longer life expectancy than probands without Lynch syndrome, because Lynch syndrome patients generally have a better prognosis for CRC than the general population.

Figure 36: Life expectancy of probands



The life expectancy of relatives is generally lower for those with Lynch syndrome since they are at increased risk of colorectal cancer and endometrial cancer (women only).

Figure 37: Life expectancy of relatives



Kaplan–Meier graphs for alternative strategies have been produced by weighting the simulated individuals in the proportions expected according to each strategy.

Figure 38 and *Figure 39* show the overall survival for probands and relatives with Lynch syndrome respectively. Kaplan–Meier graphs are not shown for individuals without Lynch syndrome as there is no separation of the survival functions.

Figure 38 shows that testing for Lynch syndrome has no immediate impact on the overall survival of probands, since it is not assumed to improve survival of the index colorectal cancer, but to reduce the risk of metachronous colorectal cancer. This is why the overall survival curves do separate after a time, when the hazard of mortality due to colorectal cancer has lowered.

Figure 38: Overall survival for probands with Lynch syndrome

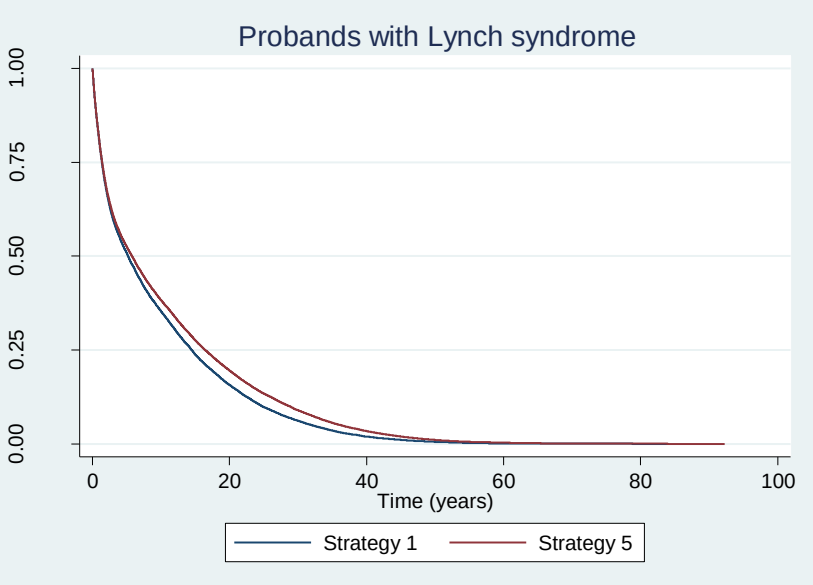
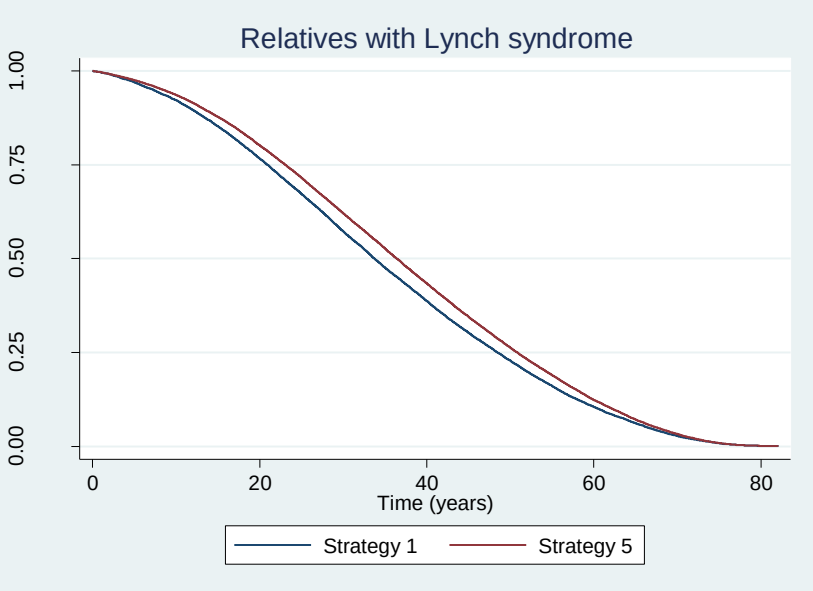


Figure 39 shows that implementing testing results in sustained improvements in overall survival of relatives with Lynch syndrome. In reality, there would be expected to be a slightly greater delay before improvement (relatives are often not diagnosed with Lynch syndrome at the same time as the proband).

Figure 39: Overall survival for relatives with Lynch syndrome



Event-free survival (i.e., the time to death, colorectal cancer incidence or endometrial cancer incidence) is also improved for probands and relatives with Lynch syndrome when a testing strategy is employed (*Figure 40* and *Figure 41*).

Figure 40: Event-free survival for probands with Lynch syndrome

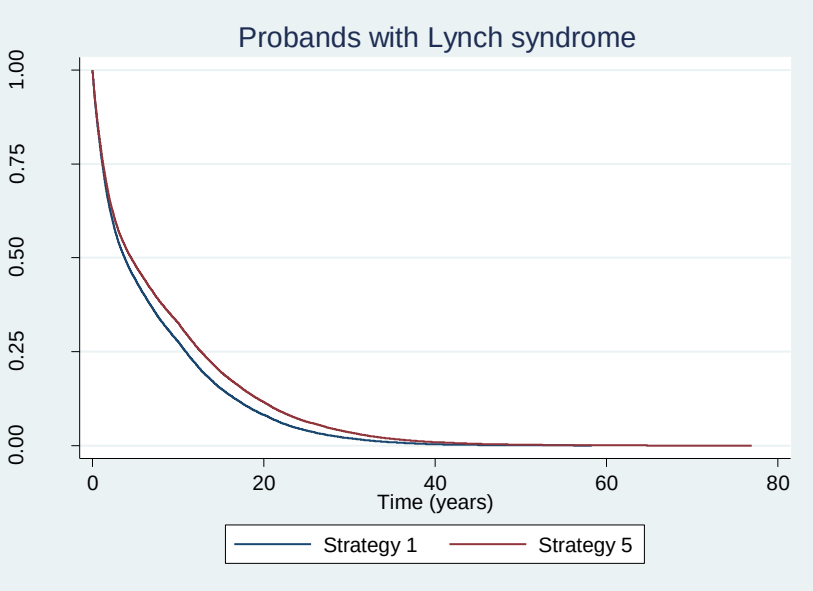
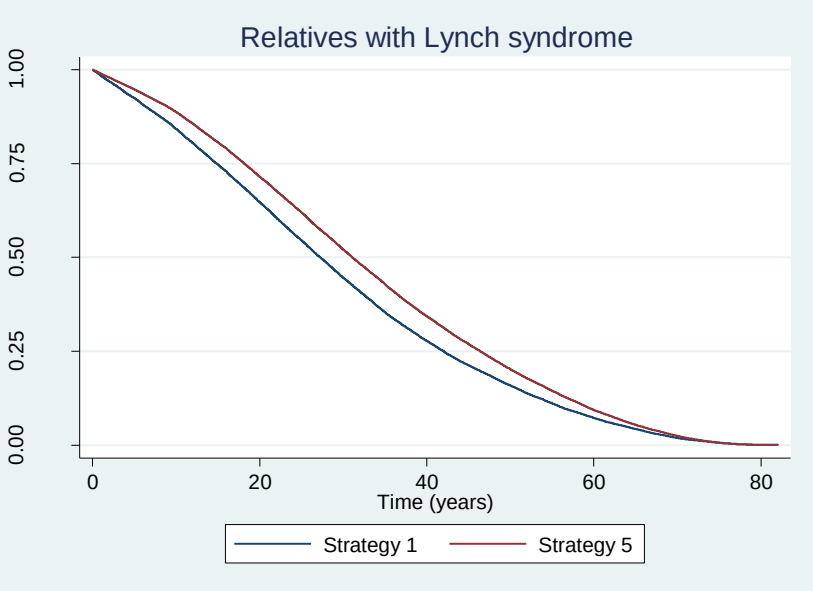


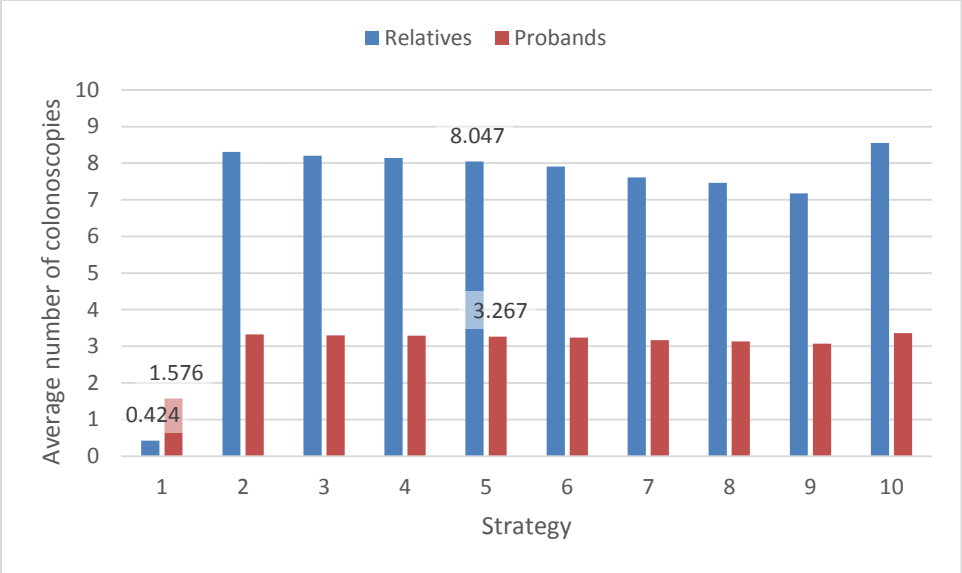
Figure 41: Event-free survival for relatives with Lynch syndrome



5.2.1.5 Additional outcomes

The average number of colonoscopies for individuals who actually have Lynch syndrome is affected significantly by the introduction of testing (*Figure 42*). In the absence of testing the expected number of colonoscopies is 0.42 for relatives and 1.58 for probands. Probands will receive 5-yearly colonoscopy as follow-up, as will relatives who develop colorectal cancer. With testing the number of colonoscopies increases significantly. Probands receive around 3 colonoscopies on average, while relatives receive 7.18 to 8.55 depending on the strategy. The variation in the number of colonoscopies here is entirely driven by the number of false negative diagnoses.

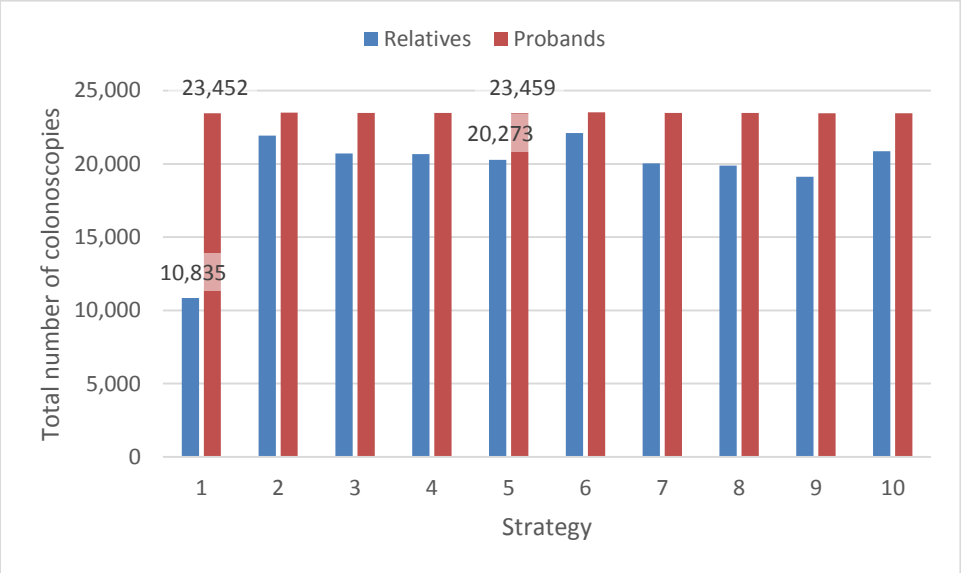
Figure 42: Average number of colonoscopies for individuals with Lynch syndrome



The average number of colonoscopies for individuals without Lynch syndrome is not significantly affected by the introduction of testing. There is a small increase in the average number of colonoscopies per relative. This small increase in the average number of

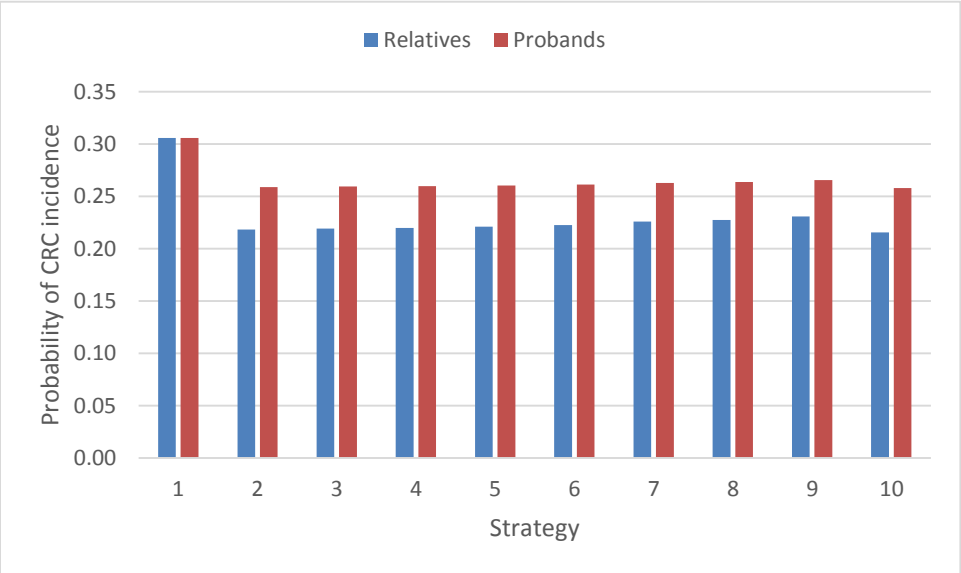
colonoscopies does, however, correspond to a large increase in the total number of colonoscopies (*Figure 43*), since relatives without Lynch syndrome comprise the largest group of modelled individuals. For each annual cohort the model predicts around 10,000 additional colonoscopies in relatives without Lynch syndrome (these may reasonably be considered unnecessary). This increase is attributable to the number of false positive diagnoses.

Figure 43: Total number of colonoscopies for individuals without Lynch syndrome



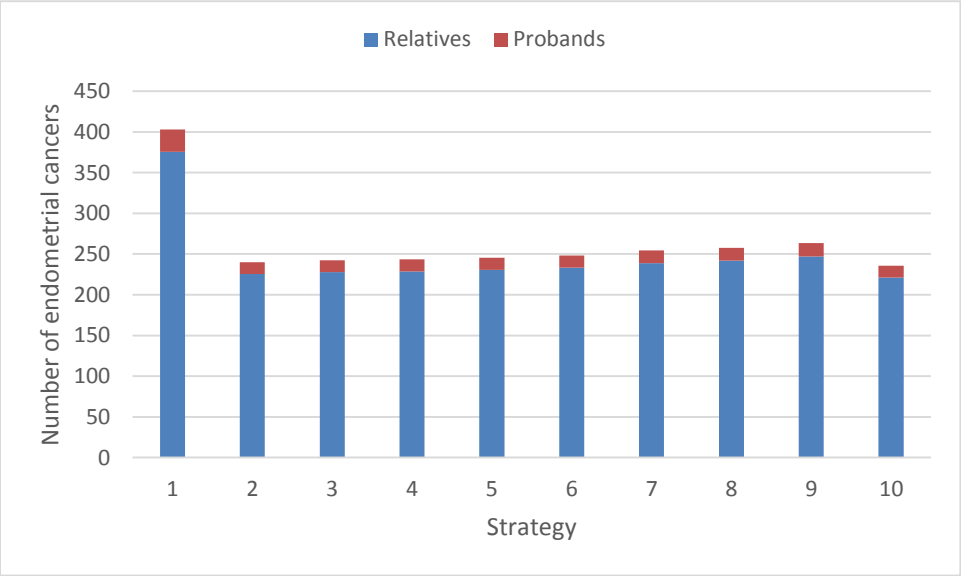
The introduction of testing for Lynch syndrome is expected to reduce the probability of subsequent CRC incidence for individuals with Lynch syndrome (*Figure 44*). There is very little impact on the probability of CRC incidence for individuals without Lynch syndrome, since testing only leads to increased surveillance in these individuals in the event of false positive diagnosis.

Figure 44: Probability of CRC incidence for individuals with Lynch syndrome



Testing is also predicted to reduce the risk of endometrial cancer for probands and relatives with Lynch syndrome (*Figure 45*). The majority of such cancers occur in relatives due to their greater numbers and life expectancy.

Figure 45: Number of endometrial cancers in individuals with Lynch syndrome



5.2.1.6 Disaggregated costs

Figure 46: Summary total undiscounted costs

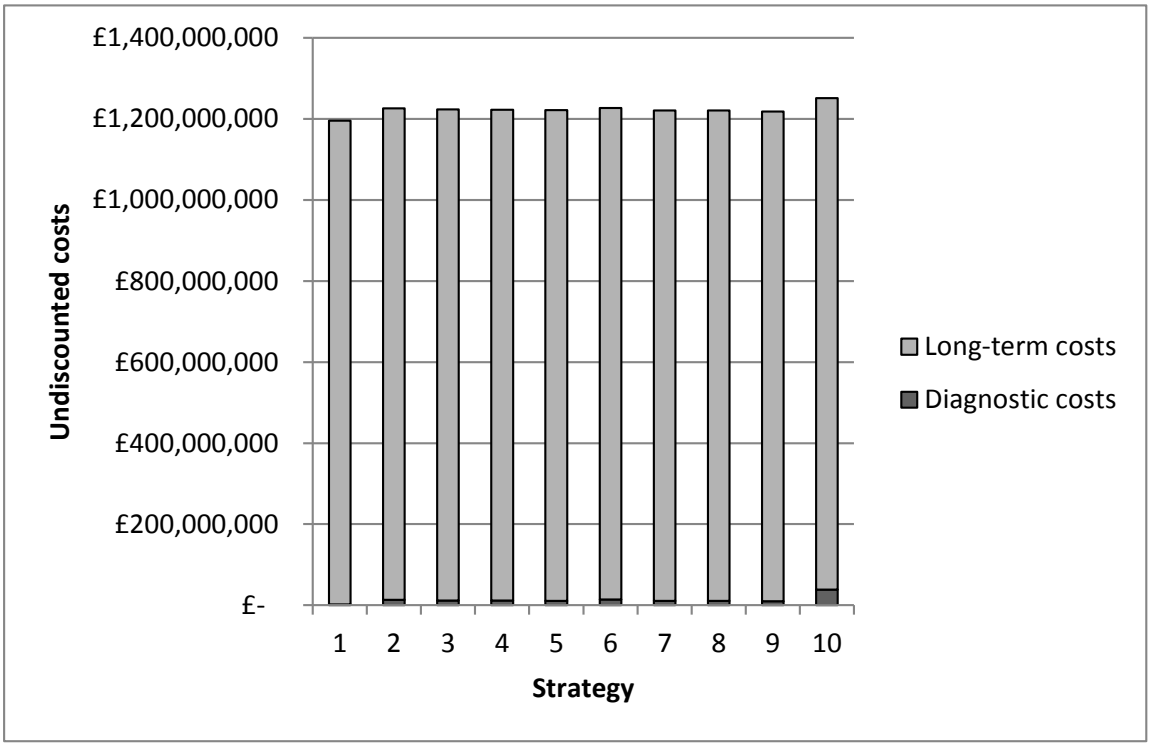
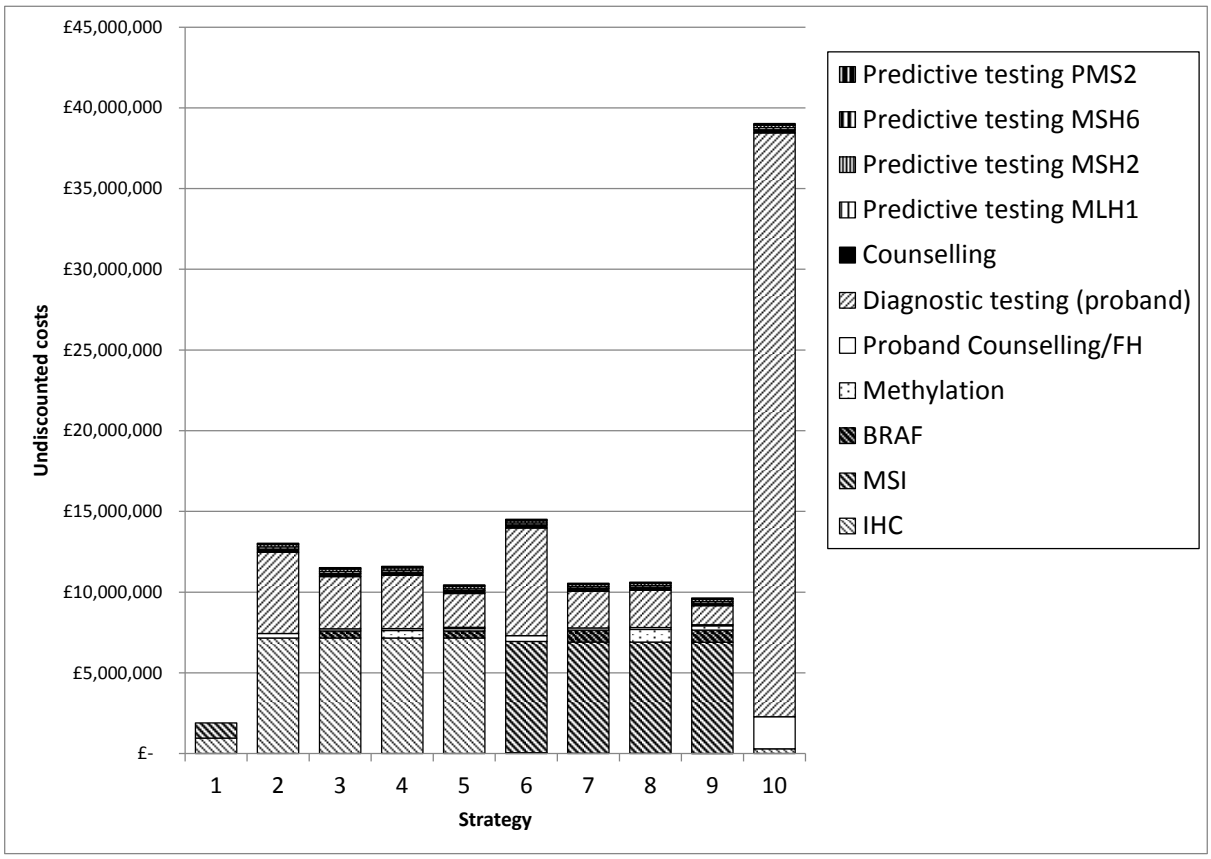


Figure 46 shows that the largest cost component for each strategy are the long term costs. However, given the small incremental difference between each arm, we see that the diagnostic costs are influential on the overall incremental costs. We explore these in more detail in *Figure 47* to *Figure 49*.

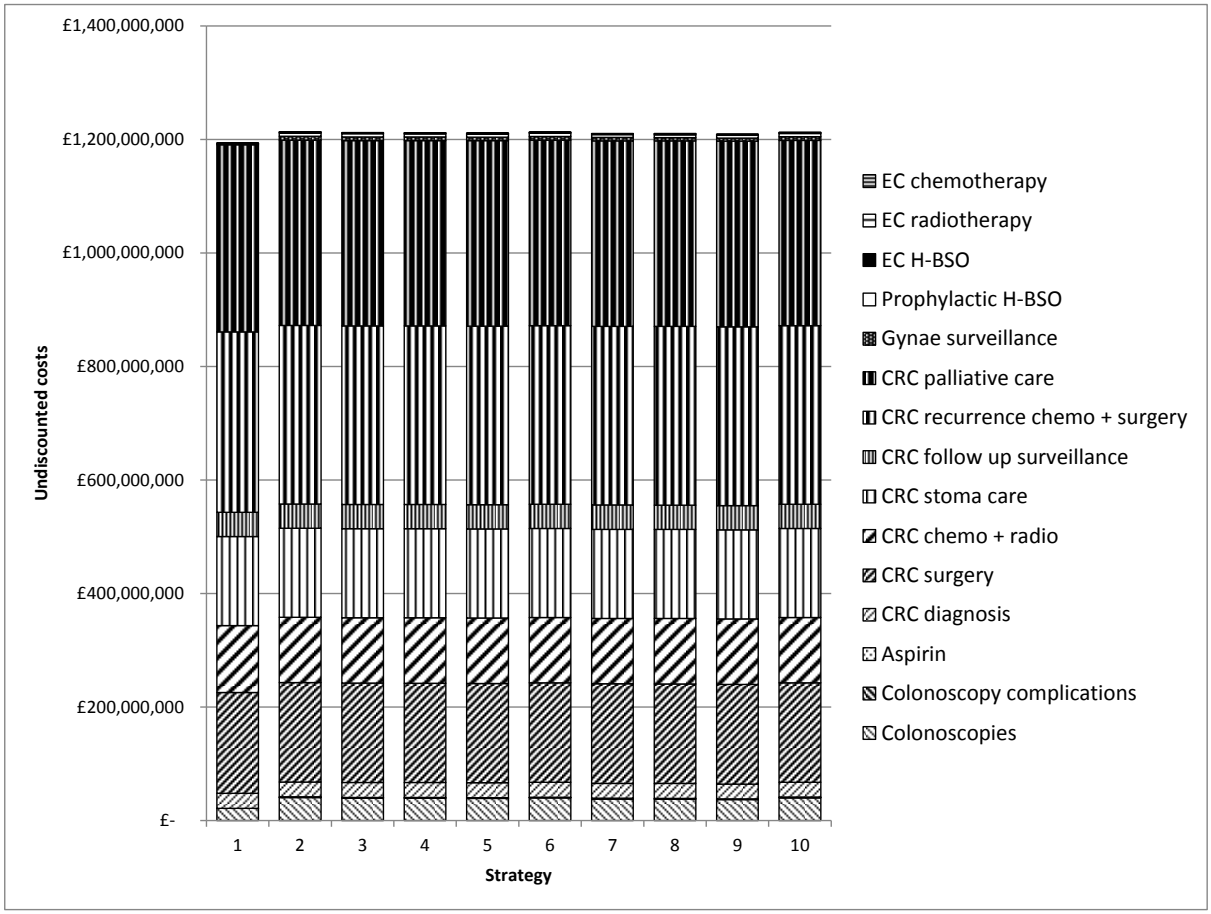
Figure 47: Undiscounted diagnostic costs, base case



Key: FH, family history

The strategy with the largest diagnostic costs is Strategy 10 (universal testing) and the least expensive is Strategy 1 (the no testing strategy). Strategy 10 is particularly expensive because genetic testing is the only test in the strategy, and genetic testing is far more expensive than IHC and MSI testing, both of which are initial tests in other strategies. In general, the MSI strategies have lower diagnostic costs, with the exception of Strategy 6, where MSI is used on its own. The larger costs here are driven by a larger proportion of probands going forward for diagnostic genetic testing. The largest cost component of every strategy is the initial test, or the cost of genetic testing for the proband.

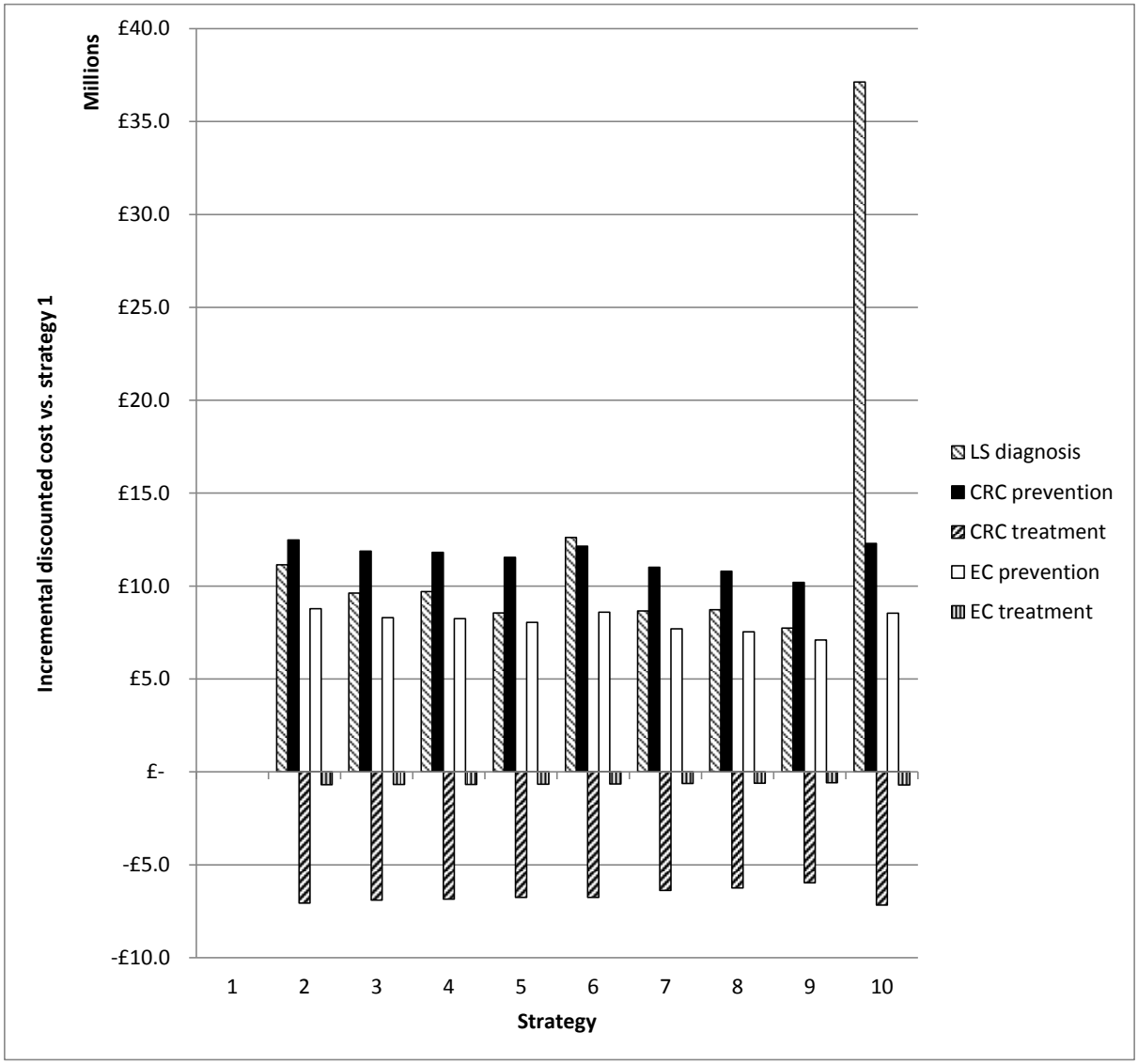
Figure 48: Long term costs, bases case



Key: CRC, colorectal cancer; EC, endometrial cancer; H-BSO, hysterectomy and bilateral salpingo-oophorectomy

Long term costs appear broadly similar across the strategies (*Figure 48*). However as Figure 49 shows, this may be because the costs of cancer prevention and costs of cancer treatment largely negate each other in each strategy, making Lynch syndrome diagnosis one of the main drivers of the incremental cost difference between strategies.

Figure 49: Incremental discounted costs versus no testing



Key: CRC, colorectal cancer; EC, endometrial cancer; LS, Lynch syndrome
Note: Cost of aspirin is included in CRC prevention costs, although it also reduces the risk of EC

5.2.2 Subgroup analyses

For subgroup analyses, a variety of age limits applied to newly-diagnosed colorectal cancer (CRC) patients (probands) are considered, as requested by the NICE Scope. When the age limit of probands is lowered, the prevalence of Lynch syndrome in probands increases (*Table 71*), because the incidence of CRC in the general population falls more rapidly than the incidence of CRC in people with Lynch syndrome. For the same reason, when the age limit is set at a minimum of 70 years, the prevalence of Lynch syndrome falls significantly (from 2.8% in base case to 1.1%). The total annual incidence of CRC also changes with each age limit and we note that there is higher incidence of CRC for people over 70 than in any other age group (20,202 compare to 13,823 in the under 70 years age group). As the number of relatives per proband does not alter, this means the overall cohort size for our analysis changes depending upon the age limit used.

All parameters that are affected by the subgroup analyses are given in *Table 71*.

As shown in *Table 72*, the mean age of the probands in the cohort alters as expected, according to the age limit of the probands, though the age of probands without Lynch syndrome is consistently estimated to be higher than that of those with Lynch syndrome. For the groups with lower maximum age limits the age of probands with and without Lynch syndrome do become more similar, reflecting the change in prevalence compared to the change in CRC incidence rates. The age of the relatives is not linked to the age of the probands, similar to the base case, and therefore the age distribution of the relatives does not alter for these analyses.

Table 71: Parameters altered in subgroup analyses

Input parameter	Base case	Age limited subgroup (years)				Source
		< 50	< 60	< 70	≥ 70	
Prevalence of LS in probands	2.8%	8.4%	5.7%	3.8%	1.1%	Hampel et al. 2008
Number of probands per annum in England	34,025	2,107	5,880	13,823	20,202	ONS Cancer Registration Statistics, England
Proportion of probands male	55.6%	51.8%	55.5%	59.2%	53.0%	ONS Cancer Registration Statistics, England 2006–14
Proportion probands assumed to have LS (tumour test results available only)	10%	21%	17%	13%	5%	Snowsill et al. (2014) model and assumptions
CRC incidence male proband without LS	0.63	0.61	0.56	0.57	0.67	ONS Cancer registration statistics, England 2013[96]
CRC incidence female proband without LS	0.72	0.70	0.66	0.68	0.75	

Key: CRC, colorectal cancer; LS, Lynch syndrome; ONS, Office for National Statistics

Table 72: Mean age of probands at diagnosis, by age subgroup

Subgroup	Mean age of probands at diagnosis (years)	
	Without Lynch syndrome	With Lynch syndrome
Base case	72.7	58.0
<50 years	41.6	39.8
<60 years	51.1	47.1
<70 years	60.0	52.3
>70 years	77.1	72.9

Summary results for the age limited subgroups are presented in *Table 73* to *Table 76*. Results on the cost-effectiveness frontier are generally similar for all subgroups (and to the base case), with Strategy 5 remaining the optimal strategy. Larger total discounted costs and

QALYs are reported for subgroups with a higher maximum age limit. This is primarily driven by the size of the cohort.

Primarily due to the higher prevalence of Lynch syndrome in subgroups with a lower age limit, the ICERs are reduced compared to the base case. For the subgroup where all probands are under 50 years old, all strategies have ICERs less than £13,000 per QALY gained compared to no testing (Strategy 1). The subgroup with a minimum age limit of 70 years old has the largest ICERs compared to no testing, and only Strategies 5 and 9 (IHC or MSI plus *BRAF* V600E and *MLH1* promoter hypermethylation testing) have ICERs below a cost-effectiveness threshold of £20,000 per QALY gained. The reason these strategies have lower ICERs is that the multiple tests in sequence have enriched the population prior to diagnostic genetic testing, reducing diagnostic costs.

Table 73: Summary cost-effectiveness results, proband maximum age 50

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	225,106	£52,979,766	—	—	—
2: IHC	225,489	£56,319,403	£8,731	215.5	£34,526
3: IHC plus *BRAF*	225,482	£56,098,834	£8,293	220.2	£19,903
4: IHC plus *MLH1* promoter methylation	225,481	£56,087,979	£8,298	219.2	Extended dominated by 5 and 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	225,476	£55,970,603	£8,090	220.1	£8,090
6: MSI	225,469	£56,333,761	£9,229	195.7	Dominated by 2
7: MSI plus *BRAF*	225,454	£55,873,734	£8,304	203.8	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	225,447	£55,831,834	£8,358	198.6	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	225,433	£55,659,876	£8,184	193.5	Extended dominated by 1 and 5
10: Universal genetic testing	225,490	£57,714,180	£12,336	147.1	£1,096,665

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Table 74: Summary cost-effectiveness results, proband maximum age 60

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	625,933	£142,438,302	—	—	—
2: IHC	626,623	£149,410,482	£10,106	341.3	£54,320
3: IHC plus *BRAF*	626,613	£148,866,574	£9,454	358.5	£25,681
4: IHC plus *MLH1* promoter methylation	626,609	£148,851,044	£9,482	355.6	Extended dominated by 5 and 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	626,601	£148,551,307	£9,156	362.0	£9,156
6: MSI	626,588	£149,549,494	£10,857	299.4	Dominated by 2
7: MSI plus *BRAF*	626,562	£148,382,032	£9,447	332.0	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	626,549	£148,307,783	£9,528	322.6	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	626,524	£147,905,444	£9,243	318.1	Extended dominated by 1 and 5
10: Universal genetic testing	626,625	£153,518,956	£16,018	137.7	£2,233,950

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Table 75: Summary cost-effectiveness results, proband maximum age 70

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	1,454,988	£322,566,730	—	—	—
2: IHC	1,456,078	£334,746,310	£11,175	480.9	£59,733
3: IHC plus *BRAF*	1,456,059	£333,636,101	£10,333	517.8	£31,707
4: IHC plus *MLH1* promoter methylation	1,456,053	£333,625,144	£10,383	512.1	Extended dominated by 5 and 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	1,456,039	£332,980,152	£9,912	529.9	£9,912
6: MSI	1,456,025	£335,222,782	£12,205	404.2	Dominated by 2
7: MSI plus *BRAF*	1,455,979	£332,749,603	£10,275	481.9	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	1,455,958	£332,649,631	£10,390	466.3	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	1,455,918	£331,857,287	£9,984	466.0	Extended dominated by 1 and 5
10: Universal genetic testing	1,456,072	£344,821,179	£20,528	−28.6	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Table 76: Summary cost-effectiveness results, proband minimum age 70

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	2,075,242	£430,082,601	—	—	—
2: IHC	2,075,648	£439,327,880	£22,794	−56.7	£146,300
3: IHC plus *BRAF*	2,075,640	£438,172,824	£20,342	−6.8	£105,987
4: IHC plus *MLH1* promoter methylation	2,075,638	£438,205,815	£20,512	−10.1	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	2,075,633	£437,415,128	£18,774	23.9	£18,839
6: MSI	2,075,629	£440,240,971	£26,305	−121.7	Dominated by 2
7: MSI plus *BRAF*	2,075,611	£437,375,080	£19,789	3.9	Extended dominated by 9 and 5
8: MSI plus *MLH1* promoter methylation	2,075,603	£437,374,272	£20,205	−3.7	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	2,075,588	£436,573,720	£18,766	21.3	£18,766
10: Universal genetic testing	2,075,621	£454,981,317	£65,701	−866.0	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

The difference in ICERs is also driven by the benefit accrued by probands within the model. Probands under 50 years old have a much higher life expectancy than probands who are over 70 years old (Figure 50 and Figure 51) and therefore the potential life years gained from diagnosing Lynch syndrome are increased the lower the age of the probands. However, there is still benefit in terms of life expectancy to proband in the >70 year subgroup.

Figure 50: Life expectancy of probands maximum age 50

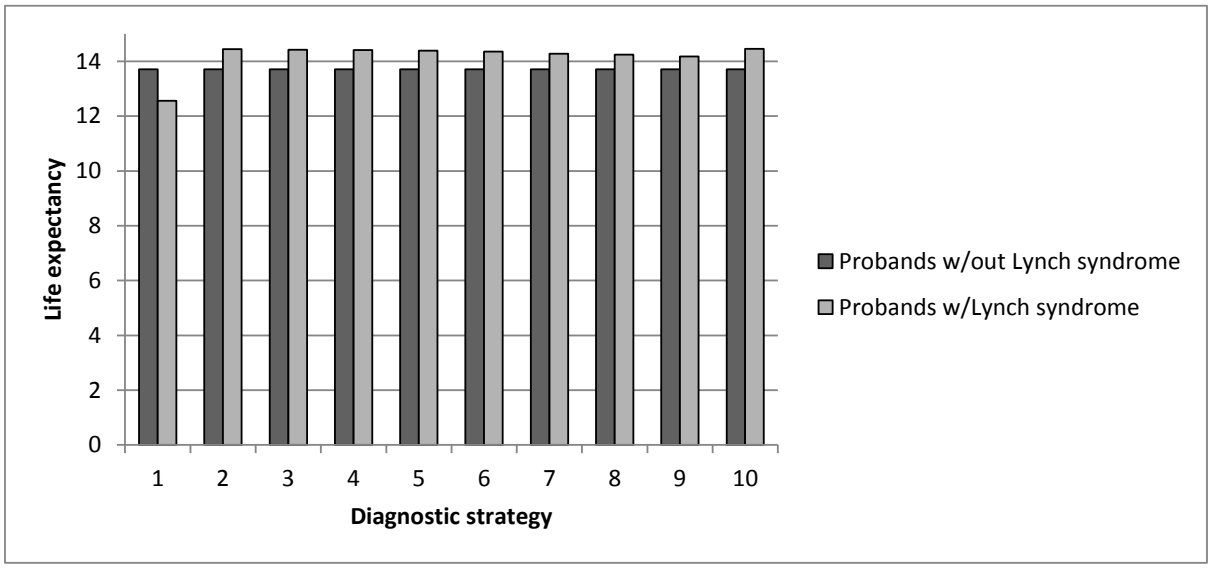
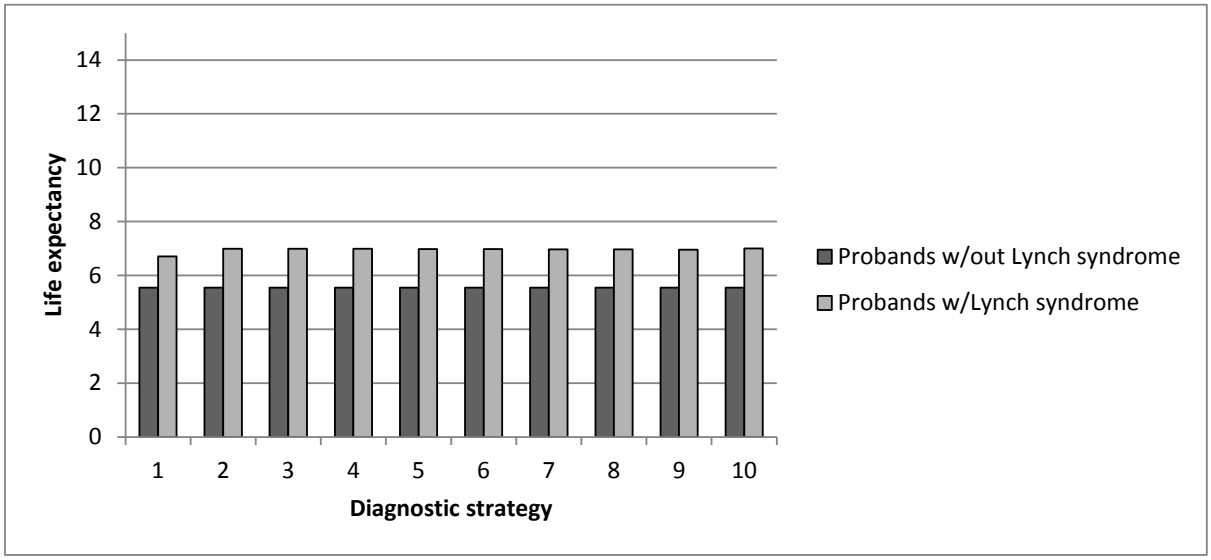


Figure 51: Life expectancy of probands minimum age 70 years



Of note, the higher total costs in the subgroups with the higher age limit are driven entirely by the larger size of the cohort. The per-person cost is reduced in the groups with a higher age limit for probands (e.g., <£5,000 for all strategies for over 70s, but >£5,900 for all strategies in the under 50s). This is primarily driven by the life expectancy of the probands in these strategies (diagnosis at an earlier age will result in higher prevention and treatment costs).

5.2.3 Scenario analyses

5.2.3.1 Scenario 1: MSI-L corresponds to a Lynch syndrome positive MSI result

As previously described, there are different thresholds which are used to decide whether a tumour has microsatellite instability. Broadly these fall into two categories: MSI-Low (MSI-L) and MSI-High (MSI-H), where MSI-H has a higher level of microsatellite instability than MSI-L (but both have some level of instability). Exact measure and cut-offs can differ according to the specific test used. Further discussion of these is given in *Section 1.2.1.1.1*.

In the base case, only MSI-H tumours are assumed indicative of Lynch syndrome. However, clinical opinion suggests that MSI-L tumours may also be used as indicative of Lynch syndrome and we explore the impact of this in this scenario analysis. As MSI-L requires a lower threshold for instability, the number of people diagnosed with microsatellite instability (MSI) increases. This has the impact of altering the accuracy of the tests as the number of true and false positives increase, whilst the number of true and false negatives decrease. *Table 77*, shows this increases sensitivity (TP/(TP+FN)) and decreases specificity (TN/(TN+FP)) compared to the base case, where MSI-H is the chosen threshold.

Table 77: Diagnostic accuracy of MSI testing, according to MSI-L or MSI-H threshold

Scenario	Sensitivity (%)	Specificity (%)
MSI = MSI-H (base case)	91.3	83.7
MSI = MSI-H or MSI-L	97.3	59.6

The cost-effectiveness results for this scenario are given in *Table 78*. As expected, the cost and QALY results for Strategies 1-5 and 10 are unaffected as MSI testing is not a part of their diagnostic pathways. For Strategies 5 to 9 both the discounted costs and QALYs have increased from the base case, however the change in cost is substantially larger than the change in QALYs (an increase of £2.5 million to £12 million compared to 110 to 160 QALYs gained, depending upon the strategy). Therefore the ICERs versus no testing for all MSI strategies have increased from the base case.

Table 78: Summary cost-effectiveness results, MSI-L is indicative of Lynch syndrome

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,508,052	£743,298,306	—	—	—
2: IHC	3,510,017	£767,955,447	£12,553	731.5	£60,967
3: IHC plus *BRAF*	3,509,977	£765,532,726	£11,553	812.9	£37,495
4: IHC plus *MLH1* promoter methylation	3,509,965	£765,535,788	£11,626	800.8	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,509,937	£764,048,240	£11,008	847.5	£11,008
6: MSI	3,510,086	£781,391,603	£18,729	129.2	£193,128
7: MSI plus *BRAF*	3,509,958	£768,160,279	£13,044	662.9	Dominated by 2
8: MSI plus *MLH1* promoter methylation	3,509,920	£768,141,508	£13,305	625.1	Dominated by 2
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,509,833	£764,450,482	£11,877	723.3	Dominated by 5
10: Universal genetic testing	3,509,987	£793,380,127	£25,884	−569.2	Dominated by 6

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

The additional costs and QALYs for the MSI strategies are driven by the increased number of probands and relatives identified as Lynch syndrome positive (*Figure 52*), leading to

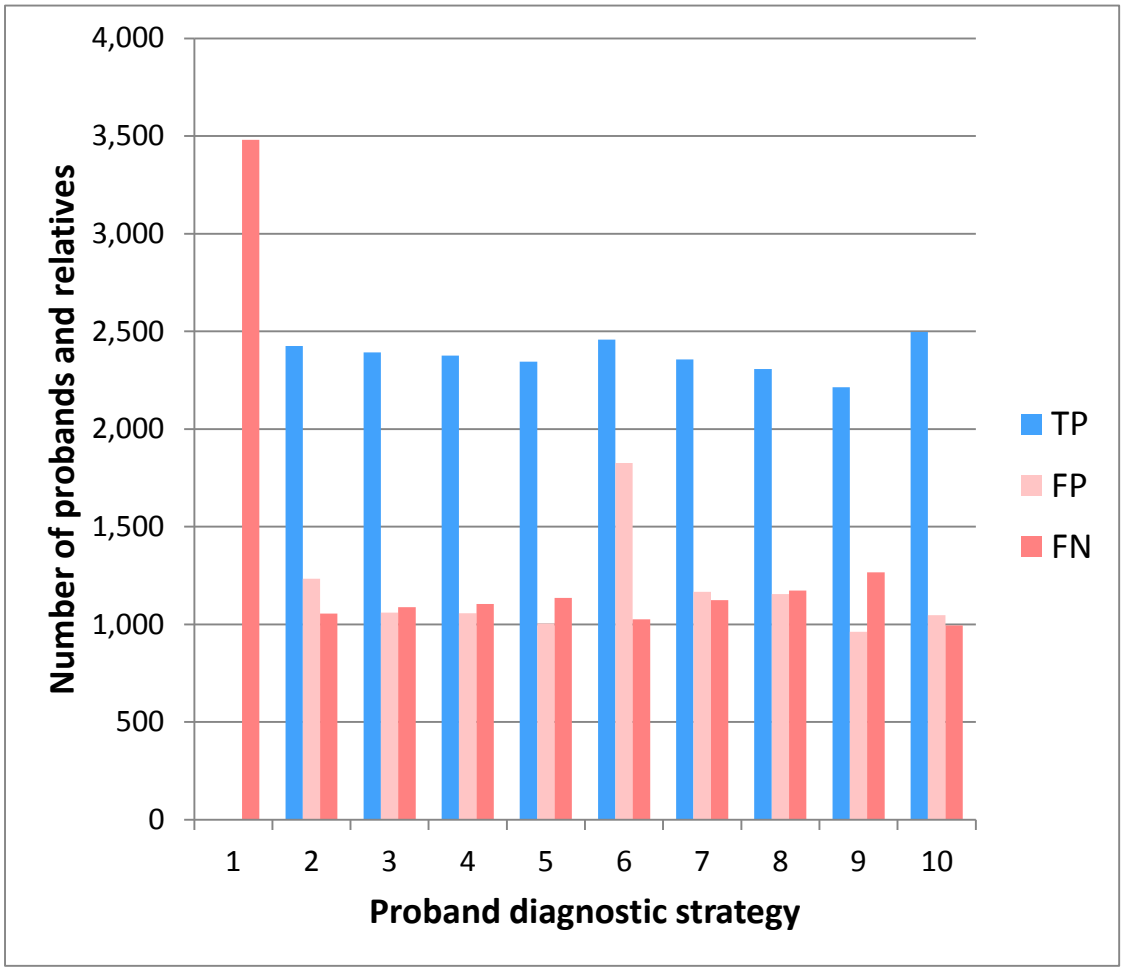
additional downstream costs and benefits associated with CRC and EC risk reductions (*Figure 54*). *Table 79* demonstrates that the sensitivity of Strategies 6–9 are now increased and specificity slightly decreased. Interestingly, in both the probands only subpopulation or in the overall population, the specificity is not greatly affected, despite the specificity of MSI testing reducing to 59%. This is a result of the downstream tests in each strategy correcting for the poorer specificity of using MSI-L as indicative of Lynch syndrome. Indeed the difference in specificity from base case falls from a difference of 0.42% (probands only) in the MSI only arm, to 0.02% when MSI is followed by both *BRAF* V600E and promoter methylation testing. Similarly the difference in sensitivity also reduces as additional tests, with imperfect sensitivity, are added to the strategy, though the difference from base case remains about 4% for all strategies.

Figure 53 demonstrates that MSI-L testing results in much higher costs of tests subsequent to MSI testing, a result of more probands receiving results indicative of Lynch syndrome. This leads to the higher overall costs of diagnosis, as demonstrated in *Figure 54*.

Table 79: Sensitivity and specificity of strategies, when MSI-L is indicative of Lynch syndrome

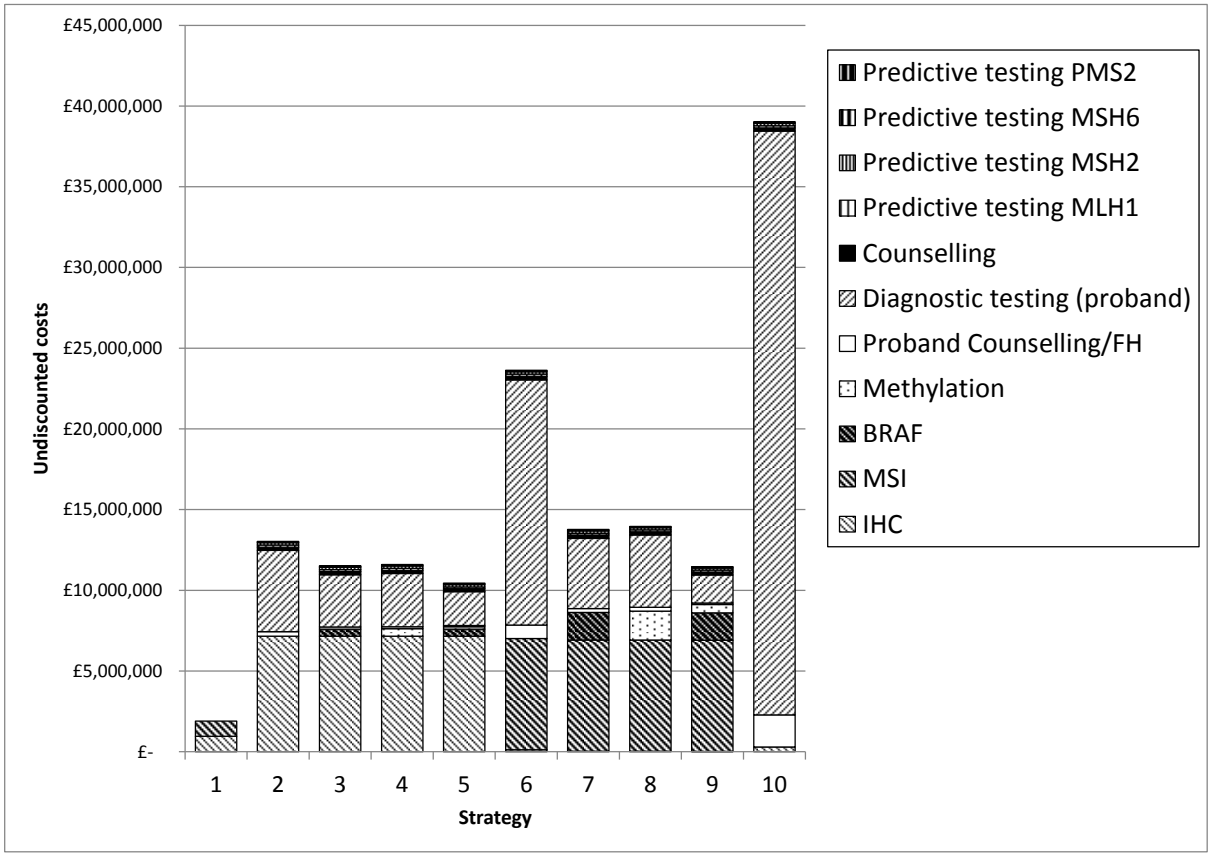
Strategy	Probands only		Overall population	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
1	0.00	100.00	0.00	100.00
2	70.35	99.80	69.67	99.47
3	69.40	99.94	68.73	99.55
4	68.93	99.93	68.26	99.55
5	68.04	99.97	67.38	99.57
6	71.19	99.30	70.54	99.22
7	68.35	99.83	67.69	99.50
8	66.92	99.83	66.28	99.51
9	64.24	99.96	63.62	99.59
10	71.43	99.98	71.53	99.55

Figure 52: Number of probands and relatives identified by each strategy, MSI-L indicative of Lynch syndrome



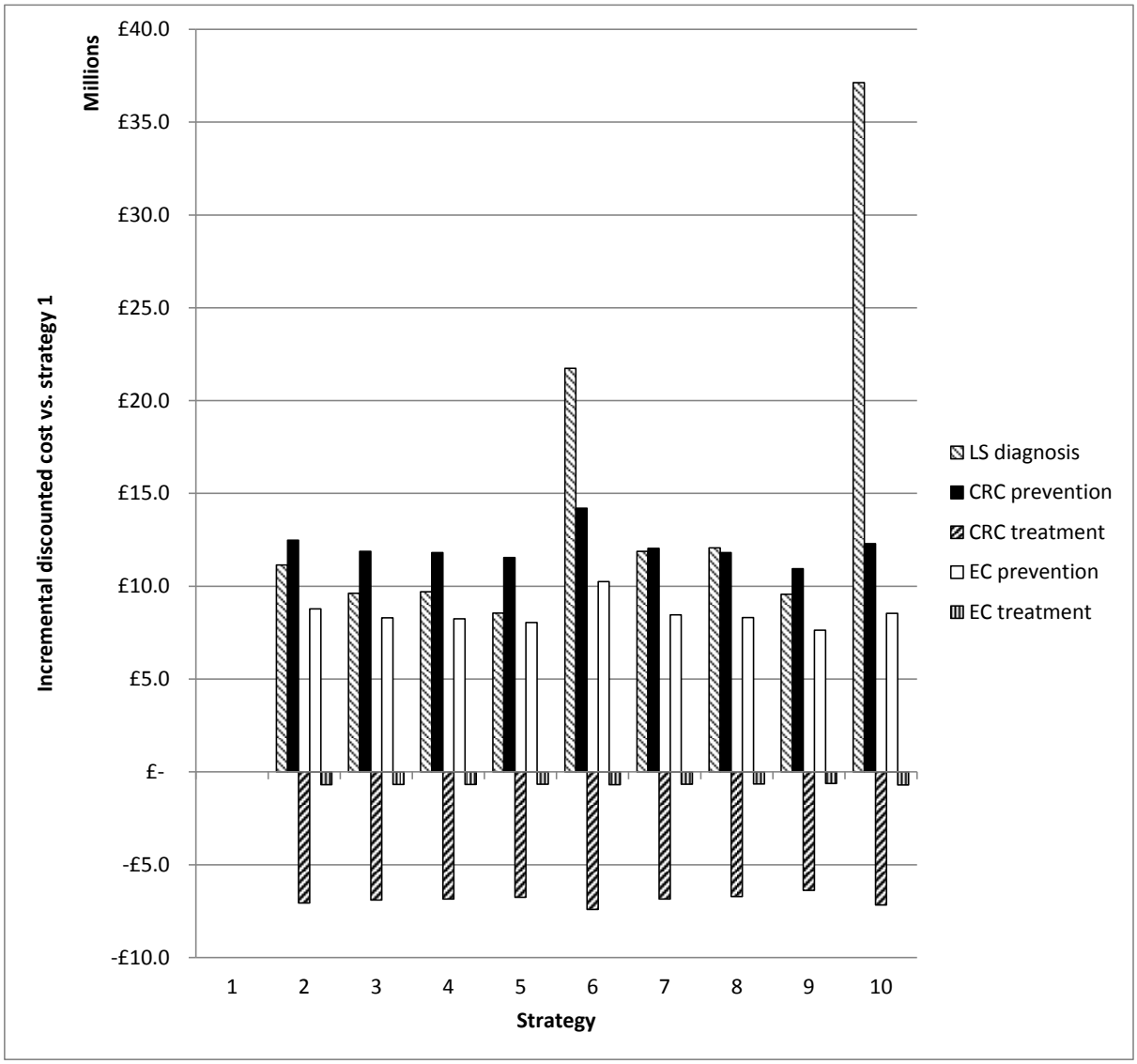
Key: FN, false negative; FP, false positive; TP, true positive
Notes: True negatives have not been shown in the interest of clarity. The number of true negatives is substantially larger than the other three diagnoses.

Figure 53: Disaggregated diagnostic costs, MSI-L indicative of Lynch syndrome



Key: FH, family history

Figure 54: Incremental discounted costs versus no testing, MSI-L indicative of Lynch syndrome



Key: CRC, colorectal cancer; EC, endometrial cancer; LS, Lynch syndrome

5.2.3.2 Scenario 2: Aspirin removed from the model

In the base case, aspirin use for CRC prevention in Lynch syndrome positive people is measured as in the CAPP2 trial. Here we explore the scenario where aspirin is instead not included as a risk reducing component of the model (and therefore remove the costs and benefits associated with its use).

There is a slight reduction in QALYs for all strategies, and in life expectancy for relatives, compared with the base case (*Figure 37* compared to *Figure 55*). However we note that the difference in life expectancy for relatives without Lynch syndrome is at least partially driven by the new set of simulations, as this holds even in Strategy 1, and is therefore likely to be related to the expected differences of running a new set of simulations. However, the life expectancy for probands and relatives with Lynch syndrome is also likely reduced by the increase in CRC and endometrial cancer incidence (though again this too has increased in Strategy 1 from the base case).

Similarly, though the cost of risk reduction has reduced, the cost of treatment for CRC and EC has increased, resulting in the higher overall costs compared to base case. Again, some of this is related to the simulations, but by comparing the incremental results of each strategy versus no testing (*Table 80*), we can see that the overall outcome of removing aspirin from the model is to marginally increase the ICERs.

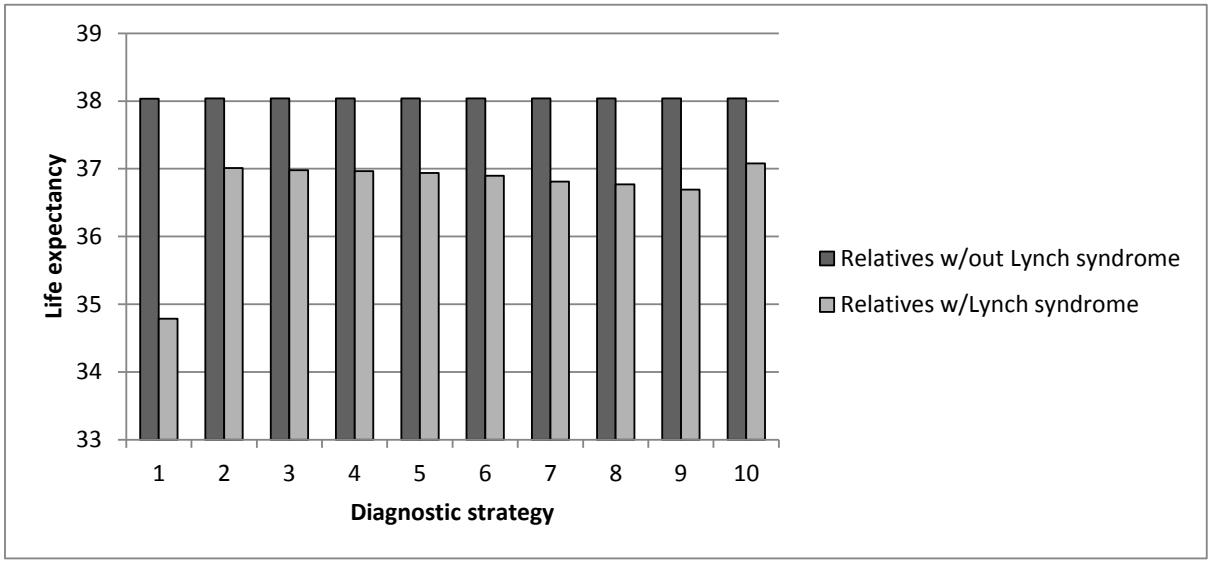
In this scenario, Strategy 5 remains the optimal strategy, with an ICER of £11,659 per QALY gained (compared to £11,008 per QALY gained in the base case).

Table 80: Summary cost-effectiveness results, aspirin removed

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,506,867	£731,729,637	—	—	—
2: IHC	3,508,703	£756,230,300	£13,350	610.2	£76,621
3: IHC plus *BRAF*	3,508,671	£753,822,657	£12,248	699.2	£41,422
4: IHC plus *MLH1* promoter methylation	3,508,660	£753,826,465	£12,326	687.8	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,508,636	£752,343,957	£11,659	737.4	£11,659
6: MSI	3,508,614	£757,522,873	£14,766	457.1	Dominated by 2
7: MSI plus *BRAF*	3,508,536	£751,959,893	£12,128	656.6	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,508,501	£751,805,586	£12,290	629.7	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,508,433	£750,096,269	£11,729	647.7	Extended dominated by 1 and 5
10: Universal genetic testing	3,508,680	£781,664,560	£27,541	−683.7	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Figure 55: Life expectancy of relatives, aspirin removed as risk reducing measure



5.2.3.3 Scenario 3: Gynaecological surveillance assumed to have no benefit

In the base case, gynaecological surveillance is offered to women diagnosed with Lynch syndrome, and if accepted incurs both a cost and reduces the risk of mortality from endometrial cancer (EnCa). However, the true benefit of this surveillance is disputed. As such, we investigate the impact of gynaecological surveillance in the next two scenarios, firstly by assuming that the surveillance has no benefit (but still incurs a cost), and secondly by removing gynaecological surveillance from the model entirely (so that there are no associated costs or benefits modelled).

In this first analysis, the HR for endometrial cancer survival is increased from 0.898 to 1, for women receiving surveillance.

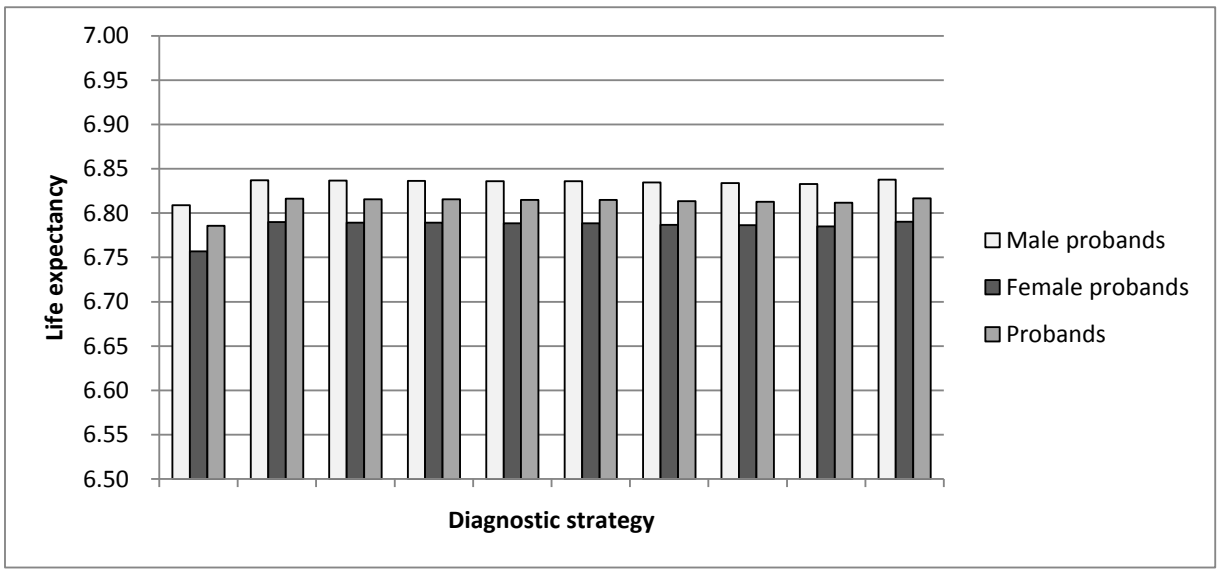
Summary results for this scenario are presented in *Table 81*. Both the incremental costs and QALYs are slightly reduced compared to the base case. Whilst a decrease in the incremental costs may seem counterintuitive (as the cost of surveillance is still incorporated in the model), this is likely offset by a reduction in the life expectancy of women in Strategies 2–10 of the model (*Figure 56* and *Figure 57*), resulting in fewer total costs and QALYs accrued over their lifetime, compared to the base case. Overall this slight reduction in incremental cost is not as pronounced as the incremental QALY loss compared to no testing and therefore the ICERs are very slightly increased compared to the base case (as expected). The optimal strategy remains Strategy 5 (IHC followed by *BRAF* and *MLH1* promoter hypermethylation testing), with an ICER of £11,375 compared to no testing.

Table 81: Summary cost-effectiveness results, no benefit assumed from gynaecological surveillance

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,518,332	£729,775,566	—	—	—
2: IHC	3,520,216	£754,377,439	£13,053	654.6	£80,413
3: IHC plus *BRAF*	3,520,186	£751,943,691	£11,954	746.0	£40,972
4: IHC plus *MLH1* promoter methylation	3,520,175	£751,947,401	£12,031	734.3	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,520,150	£750,457,773	£11,375	784.1	£11,375
6: MSI	3,520,124	£755,682,001	£14,453	497.2	Dominated by 2
7: MSI plus *BRAF*	3,520,046	£750,076,890	£11,839	699.7	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,520,011	£749,921,886	£11,998	671.8	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,519,942	£748,200,353	£11,441	689.2	Extended dominated by 1 and 5
10: Universal genetic testing	3,520,197	£779,782,852	£26,815	−635.5	Dominated by 2

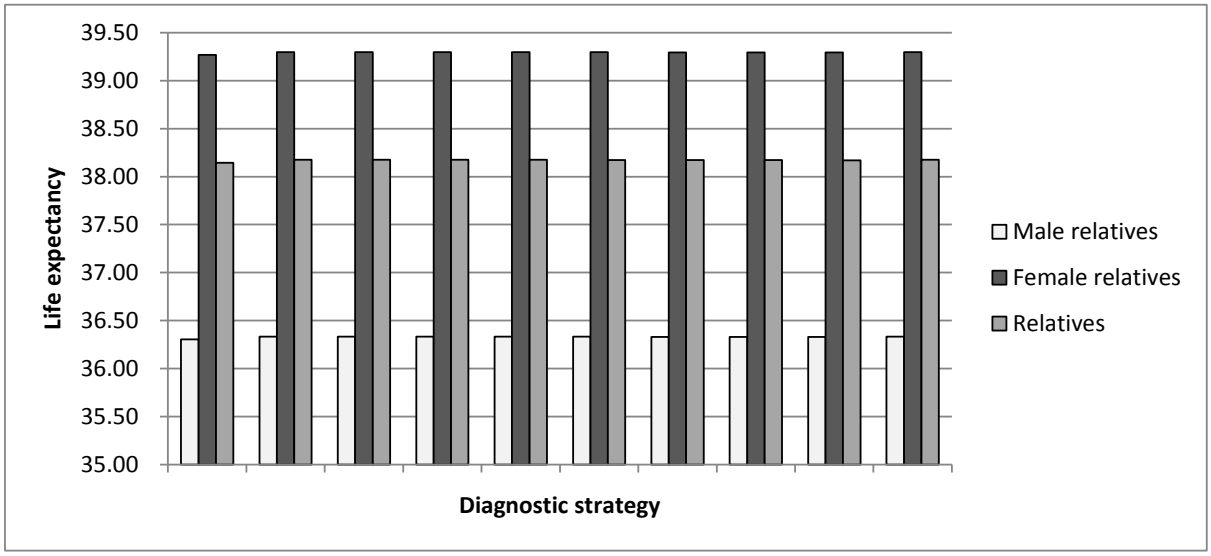
Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Figure 56: Life expectancy, probands, gynaecological surveillance has no benefit



Note: The increase in life expectancy for male probands is a result of the simulation run and appears more pronounced based on the scale of the figure.

Figure 57: Life expectancy, relatives, gynaecological surveillance has no benefit



5.2.3.4 Scenario 4: Gynaecological surveillance not included

As described in *Section 5.2.3.3*, this scenario removes all gynaecological surveillance from the model (i.e., the probability of being offered gynaecological surveillance becomes 0).

This scenario results in similar incremental QALY gains versus Strategy 1 as Scenario 3 (absolute QALY gains differ due to a different simulation run), but additional reductions in incremental costs due to the removal of the cost of gynaecological surveillance. The resulting ICERs versus no testing are slightly reduced compared to both Scenario 3 and the base case, with the exception of universal genetic testing (Strategy 10), which has a larger ICER than in the base case. This strategy differs as the change in surveillance costs is less influential on the overall incremental costs, given that the cost of diagnosing Lynch syndrome is the main driver of the overall incremental costs versus no testing (*Figure 58*).

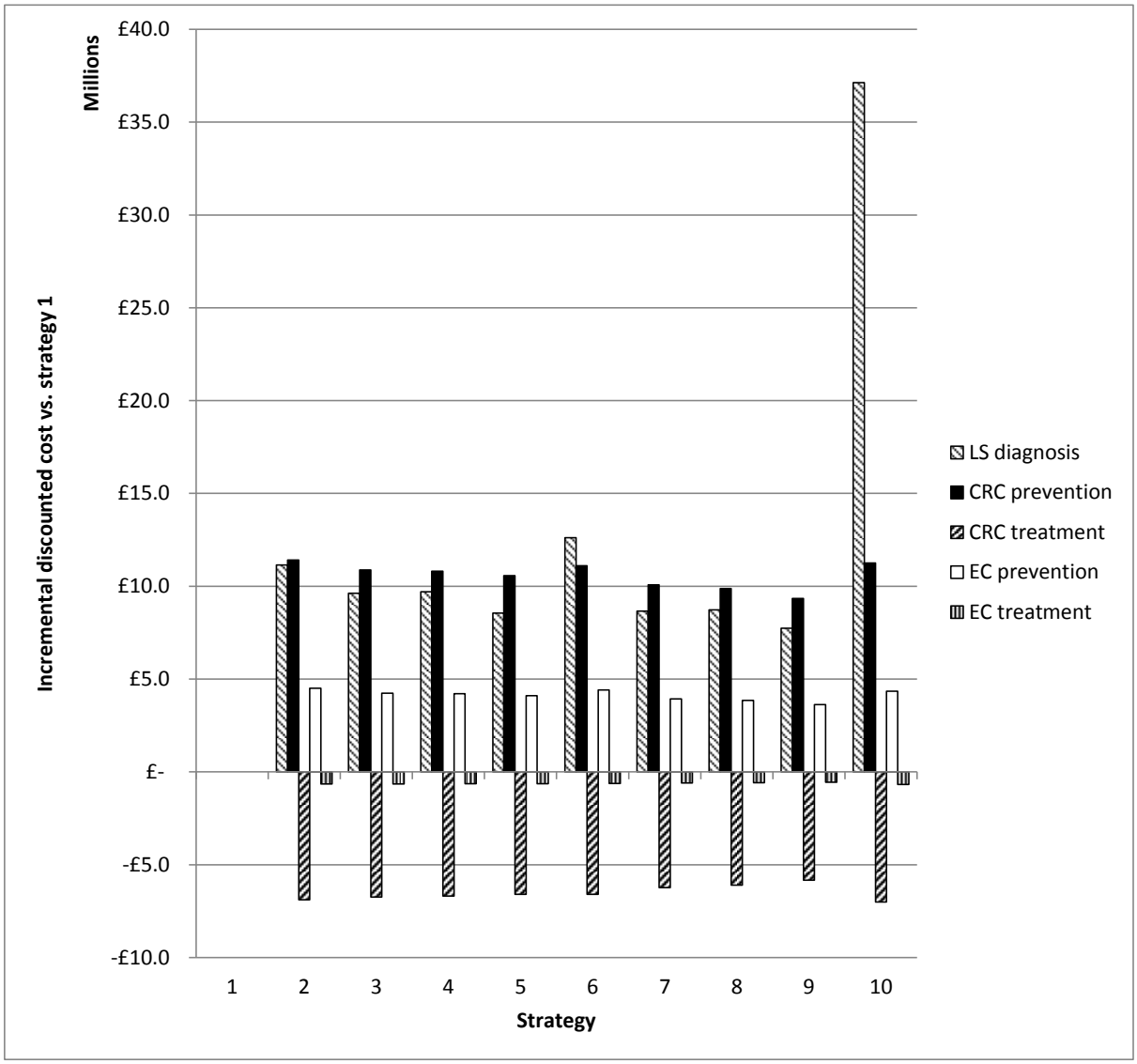
As with Scenario 3, Strategy 5 remains the optimal strategy, with an ICER of £10,241 per QALY gained.

Table 82: Summary cost-effectiveness results, no gynaecological surveillance

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,524,850	£729,116,320	—	—	—
2: IHC	3,526,553	£749,618,872	£12,033	678.8	£109,979
3: IHC plus *BRAF*	3,526,533	£747,412,582	£10,866	769.1	£44,025
4: IHC plus *MLH1* promoter methylation	3,526,523	£747,439,747	£10,950	757.2	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,526,502	£746,042,546	£10,241	806.4	£10,241
6: MSI	3,526,465	£751,010,931	£13,553	520.7	Dominated by 2
7: MSI plus *BRAF*	3,526,406	£745,828,322	£10,736	721.0	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,526,374	£745,742,636	£10,909	692.7	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,526,314	£744,227,411	£10,318	709.0	Extended dominated by 1 and 5
10: Universal genetic testing	3,526,539	£775,139,777	£27,239	−611.6	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Figure 58: Incremental discounted costs versus no test, no gynaecological surveillance



Key: CRC, colorectal cancer; EC, endometrial cancer; LS, Lynch syndrome

5.2.3.5 Scenario 5: CRC utilities taken from Ness et al. (1999)

In the base case, quality of life (as measured by the EQ-5D) in all CRC stages, except Dukes' D, is expected to be similar to those of the general population. Dukes' Stage D CRC is expected to have a non-zero disutility of 0.13. Our review of CRC utilities provided some evidence to support this approach. However, it is important to investigate a scenario where all stages of CRC incur a quality of life decrement, to assess the impact this has upon the cost-effectiveness results. Ness et al. (1999),[134] which reports CRC utilities, has been widely cited in previous cost-effectiveness analyses of CRC (including Snowsill et al., 2014) and is therefore chosen for this scenario analysis. A comparison of the disutilities between base case and scenario analysis is given in Table 83.

Table 83: CRC disutility parameters

Stage	Base case disutilities[132, 133]	Scenario analysis disutilities[134]
Dukes A	0	0.11
Dukes B	0	0.23
Dukes C	0	0.26
Dukes D	0.13	0.60

A summary of the results are given in *Table 84*. As expected, the ICERs for Strategies 2-10 versus no testing are reduced compared to the base case. Incremental cost differences compared to Strategy 1 remain broadly similar (again absolute cost gains differ from the base case due to the set of simulation). However, the incremental QALYs gained versus no testing have increased from the base case; for example, incremental QALYs in Strategy 5 increase from 1,885 in the base case to 2,116 QALYs gained versus no testing in this scenario. This reflects the additional benefit of reducing CRC incidence in probands and relatives diagnosed with Lynch syndrome (i.e., the avoidance of quality of life loss associated with CRC).

Strategy 5 remains the optimal strategy, with an ICER of £9,775 per QALY gained versus no testing.

Table 84: Summary cost-effectiveness results, CRC disutilities increased

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,459,708	£729,043,142	—	—	—
2: IHC	3,461,906	£753,654,444	£11,195	967.9	£62,734
3: IHC plus *BRAF*	3,461,867	£751,217,817	£10,268	1,050.9	£34,368
4: IHC plus *MLH1* promoter methylation	3,461,854	£751,221,527	£10,335	1,037.1	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,461,824	£749,731,129	£9,775	1,081.9	£9,775
6: MSI	3,461,801	£754,960,430	£12,380	797.5	Dominated by 2
7: MSI plus *BRAF*	3,461,705	£749,350,705	£10,169	981.6	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,461,663	£749,195,658	£10,305	948.0	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,461,582	£747,472,833	£9,833	952.8	Extended dominated by 1 and 5
10: Universal genetic testing	3,461,891	£779,058,290	£22,904	−317.1	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

5.2.3.6 Scenario 6: Colonoscopic surveillance assumed to have no impact on CRC incidence

As discussed in *Colorectal cancer incidence* (*page 184*) there is some evidence suggesting that our base case assumption for the effectiveness of colonoscopy upon CRC incidence may be optimistic. We therefore investigate a 'worst case' scenario where the hazard ratio (HR) of CRC incidence whilst receiving colonoscopic surveillance is set to 1 for both index and metachronous colorectal cancers (i.e., surveillance has no impact on CRC incidence). Previously these were set as 0.387 for index cancers (applicable to only relatives) and 0.533 for metachronous cancers (applicable to probands and relatives). Costs of colonoscopic surveillance remain as in the base case.

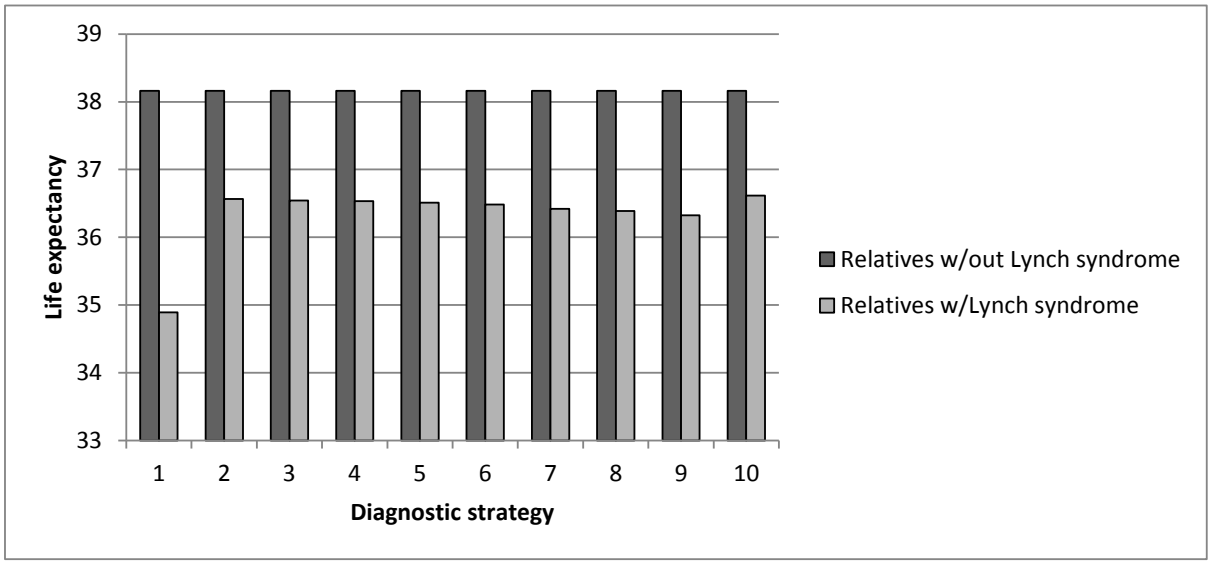
Summary results are presented in *Table 85*. As expected, as the ICERs have increased for all strategies, resulting from lower QALYs in Strategies 2–10 than in the base case, but similar costs. This lower QALY gain is driven by the increase in CRC incidence for these arms, and the resulting reduction in life expectancy. This is seen particularly for relatives (*Figure 59* compared to *Figure 37*), who in the base case receive the most benefit from colonoscopic surveillance (particularly as the base case HR for index CRC incidence with colonoscopic surveillance is lower than for metachronous CRC).

Table 85: Summary cost-effectiveness results, colonoscopic surveillance does not affect CRC incidence

Strategy	QALYs	Cost	ICER vs. no testing (cost per QALY)	INHB £20k/QALY vs. no testing	Incremental ICER (cost per QALY)
1: No test	3,516,035	£734,308,670	—	—	—
2: IHC	3,517,362	£763,001,311	£21,613	−107.1	£125,265
3: IHC plus *BRAF*	3,517,342	£760,495,764	£20,028	−1.8	£62,975
4: IHC plus *MLH1* promoter methylation	3,517,334	£760,472,422	£20,131	−8.5	Dominated by 3
5: IHC plus *BRAF* and *MLH1* promoter methylation	3,517,317	£758,927,199	£19,194	51.7	£19,194
6: MSI	3,517,296	£764,111,396	£23,625	−228.7	Dominated by 2
7: MSI plus *BRAF*	3,517,244	£758,324,753	£19,861	8.4	Extended dominated by 8 and 5
8: MSI plus *MLH1* promoter methylation	3,517,219	£758,092,893	£20,087	−5.2	Extended dominated by 9 and 7
9: MSI plus *BRAF* and *MLH1* promoter methylation	3,517,171	£756,219,424	£19,284	40.7	Extended dominated by 1 and 5
10: Universal genetic testing	3,517,331	£788,502,246	£41,796	−1,413.0	Dominated by 2

Key: ICER, incremental cost-effectiveness ratio; INHB, incremental net health benefit; QALY, quality-adjusted life year

Figure 59: Life expectancy of relatives, colonoscopy assumed to not reduce CRC incidence



5.2.4 Deterministic sensitivity analyses

Here we present the deterministic sensitivity analyses. Summary results of all analyses can be found in *Table 86*, and the subset that most influences the results are discussed further in the text. In the table we report the incremental net health benefit versus no testing for the optimal strategy, and report which strategy this refers. This provides a more meaningful comparison than an isolated ICER. Net health benefit is defined as the total QALYs minus (total costs divided by the willingness-to-pay threshold) of each strategy. Strategies with the highest NHB are found to be the optimal strategy for a chosen willingness to pay threshold. In real terms, as reported in Snowsill et al. (2014): "the total discounted QALYs for a man who lives to age 80 years, allowing for age-related quality of life, is approximately 25".

The deterministic sensitivity analyses demonstrate that the model is sensitive to several parameters.

Firstly, diagnostic accuracy of the strategies has an impact on the order of the strategies. To investigate the impact of diagnostic test performance we considered several scenarios: one where we altered the sensitivity of all tumour tests, one where we altered the specificity of all tumour tests and one where we altered the sensitivity and specificity of all tests. The values of the analyses were based on the reported or estimated 95% CIs for the tests. The aim of these analyses is to demonstrate the impact of diagnostic accuracy on the results and not necessarily to reflect what we believe to be a true reflection of current practice. *Table 86* clearly shows that reducing sensitivity and specificity (either individually or jointly) not only reduces the INHB of those Strategies 2–9 (Strategy 10 remains unchanged as the diagnostic accuracy of gene testing is unchanged), but in the case of reduced sensitivity, Strategy 4 (IHC followed by *MLH1* promoter methylation) becomes the optimal strategy affects which strategy is optimal in terms of cost-effectiveness. This is likely due to the combination of fewer diagnostic costs (less people diagnosed Lynch syndrome positive at each stage) and higher overall sensitivity than Strategy 5. When both sensitivity and specificity of each tumour test are reduced to the lowest values, the ICER for Strategy 5 versus no testing increases to £16,036 per QALY.

When sensitivity is increased for all tests, strategies with MSI testing become optimal (Strategy 9 when just altering sensitivity, Strategy 7 when increasing both sensitivity and specificity). This occurs despite the MSI strategies still having worse diagnostic outcomes in comparison to the IHC arms in terms of overall sensitivity and specificity, and is the result of a combination of factors, including additional costs in the IHC arms from identifying a higher rate of true positives not being entirely offset by the additional benefits.

Acceptance of genetic tests by probands also influences the cost-effectiveness results. In the base case, acceptance of genetic testing is relatively high (90% acceptance of tests following genetic counselling, where acceptance of counselling was 92.5%). By setting both of these parameters to 50%, the ICER for Strategy 5 versus no testing increases to £17,767 per QALY gained. One of the reasons this analysis is likely to not have produced an ICER over £20,000 per QALY gained is that a proportion of probands are assumed to have Lynch syndrome and therefore still receive CRC and endometrial cancer risk reducing measures.

However, the diagnosis of LS assumed is also shown to greatly impact the results. If all LS assumed probands and relatives are instead diagnosed as Lynch syndrome negative, the ICER for Strategy 5 decreases significantly to £5,225 per QALY gained. This is likely because the number of false positives in the Lynch syndrome assumed population is similar to the number of true positives in the base case (48% to 65% depending upon the strategy), which increases risk reduction costs without a significant benefit.

Of the diagnostic costs, IHC and MSI have the biggest impact on results, as they are the most significant drivers of the overall diagnostic cost (as they are applied to all probands in the relevant arms). They also affect the costs of Strategy 1, where MSI and IHC are costed for to reflect current practice and are incorporated into the costs of diagnosing CRC. For all analyses adjusting the cost of IHC or MSI, the ICERs remain below the £20,000 per QALY cost-effectiveness threshold.

As in Snowsill et al. (2014), only including probands in the model increases the ICERs of all strategies (Strategy 5 versus no testing increase to £17,921 per QALY gained) and reduces the INHB versus no testing. Increasing the number of relatives to 12 decreases the ICERs as expected (the size of the cohort who can benefit from CRC and endometrial cancer risk reduction increases), but the impact does not appear as significant (ICER of Strategy 5 versus no testing decreases to £10,068 per QALY gained).

The model is also sensitive to CRC incidence for people with Lynch syndrome. At high incidence the ICER for Strategy 5 versus no testing decreases to £6,689 per QALY gained and low CRC incidence the ICER increases to £19,300 versus no testing. This is expected, as lower CRC incidence results in less benefit from the risk reducing measures.

The model is also sensitive to the cost of colonoscopy, particularly increases to this cost. When cost of colonoscopy is doubled, all ICERs increase versus no testing, with the ICER for Strategy 5 increasing to £16,630 per QALY gained.

One other parameter that impacts results is the disutility associated with prophylactic H-BSO. In the base case, the disutility is assumed to be 0. In sensitivity analysis we increase this to 0.04 for 1 year, matching the disutility of endometrial cancer. This decreases the INHB and increases the ICERs for all strategies versus no testing, with the ICER for Strategy 5 versus no testing increasing to £14,441 per QALY gained. As the disutility for prophylactic H-BSO is relatively uncertain (no literature was identified to provide estimates), it is important to recognise the impact this parameter can have upon the model.

The results are also moderately sensitive to acceptance of colonoscopic surveillance. Reducing the acceptance to surveillance from over 90% for people with confirmed Lynch syndrome mutation status to 70% (the acceptance rate for people assumed to have Lynch syndrome), the INHB reduces and ICERs versus no testing increase. Though we were unable to assess the acceptance of gynaecological surveillance in the same way, it is likely to affect the results in a similar manner, as less Lynch syndrome positive people will be receiving risk reducing measures.

All other deterministic sensitivity analyses do not appear to significantly alter the cost-effectiveness results, when applied in isolation.

Table 86: Deterministic sensitivity analyses

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
Diagnostic parameters					
LS positive population by gene	*MLH1* 32% *MSH2* 39% *MSH6* 14% *PMS2* 15%	*MLH1* 40% *MSH2* 46% *MSH6* 11% *PMS2* 2%	889.2		
Proportion of probands require IHC at time of genetic test (MSI strategies only)	5%	0%	847.5		
		10%	847.5		
Diagnostic test performance					
Sensitivity	MSI 0.913 IHC 0.962 *BRAF* V600E 0.96 *MLH1* promoter methylation 0.94	Lower 95% CI MSI 0.426 IHC 0.694 *BRAF* V600E 0.60 *MLH1* promoter methylation 0.79	381.8	Strategy 4 (IHC and methylation)	414.6
		Upper 95% CI MSI 0.993 IHC 0.996 *BRAF* V600E 0.99 *MLH1* promoter methylation 0.98	920.3	Strategy 9 MSI followed by *BRAF* and methylation	924.1
Specificity	MSI 0.837 IHC 0.884 *BRAF* V600E 0.76 *MLH1* promoter methylation 0.75	Lower 95% CI MSI 0.0.638 IHC 0.790 *BRAF* V600E 0.60 *MLH1* promoter methylation 0.59	696.4		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
		Upper 95% CI MSI 0.937 IHC 0.940 *BRAF* V600E 0.87 *MLH1* promoter methylation 0.86	901.3	Strategy 3 IHC followed by *BRAF* V600E	906.9
Sensitivity and specificity	As above	Lower 95% CI, both sensitivity and specificity	230.7		
		Upper 95% CI, both sensitivity and specificity	974.2	Strategy 7 MSI followed by *BRAF*	986.8
Acceptance of genetic testing probands	Genetic counselling 92.5% Genetic test| GC 90%	Genetic counselling 100% Genetic test| GC 100%	1,105.9		
		Genetic counselling 50% Genetic test| GC 50%	74.3		
Relatives	Genetic counselling 77.7% Genetic test| GC 71.6%	Genetic counselling 100% Genetic test| GC 100%	1,070.0		
		Genetic counselling 50% Genetic test| GC 50%	670.2		
Proportion of probands LS assumed (following declined GT)	10%	0%	838.3		
		20%	856.4		
No LS assumed (no confirmed mutation= LS negative)	Probands who decline testing (and their relatives) and relatives who decline testing	Only confirmed mutation status is treated as LS positive	1,291.1		
Number of relatives	6	0	46.9		
		12	1,648.1		
Psychological disutility of	Declining testing 0.04	0 for all	883.4		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
testing	Testing LS positive 0.02	Declining testing 0.12 Testing LS positive 0.06 (equivalent to lasting a year)	775.6		
Diagnostic costs					
IHC	£210	£105	1,002.3		
		£420	537.9	Strategy 7 MSI followed by *BRAF*	809.3
MSI	£202	£101	824.3	Strategy 7 MSI followed by *BRAF*	911.0
		£405	893.9		
BRAF	£119	£60	858.6		
		£238	825.3		
Methylation	£125	£62	850.9		
		£249	840.6		
GC probands	£63	£32	849.7		
		£127	843.1		
GT probands	All four genes £1,276 *MLH1* £481, *PMS2* £468 (only applied to Strategy 3,4 and 5)	All four genes £610 (Cheapest testing option)	898.9		
GC for relatives	£63	£32	852.3		
		£127	830.2		
Testing relatives	*MLH1* £166 *MSH2* £161 *MSH6* £161 *PMS2* £165	*MLH1* £83 *MSH2* £80 *MSH6* £81 *PMS2* £83	856.1		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
		MLH1 £331 *MSH2* £321 *MSH6* £322 *PMS2* £330	820.6		
CRC parameters					
Acceptance of CRC surveillance	LS mutation 97.2% LS assumed 70.1% (probands and relatives)	LS mutation 70.1% LS assumed 70.1%	593.6		
		LS mutation 97.2% LS assumed 97.2%	948.1		
Logistic model parameters for CRC incidence in individuals with Lynch syndrome	β_0 Male 0.464 Female 0.435	β_0 Male 0.303 Female 0.265	41.5		
		β_0 Male 0.715 Female 0.697	1,770.2		
CRC incidence, HR for LS survival	Dukes A and B: 0.57 Dukes C and D: 1	Dukes A, B, C, D: 1	843.9		
CRC surgery disutility	Segmental resection 0 Subtotal colectomy IRA 0 Rectal excision 0 Proctocolectomy 0	Segmental resection 0 Subtotal colectomy IRA 0.1 Rectal excision 0.1 Proctocolectomy 0.1	875.3		
CRC related costs		**Halved and doubled**			
Colonoscopy	£585.80	£292.9	1,112.4		
		£1,171.6	317.6		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
Colonoscopy complication	Bleeding £473-£4,394 (severity dependent) Perforation or mortality £4,909	Bleeding £237-£2,197 (severity dependent) Perforation or mortality £2,455	848.9		
		Bleeding £947-£8,788 (severity dependent) Perforation or mortality £9,818	844.8		
Aspirin	£149	£74	870.0		
		£297	802.5		
CRC diagnosis	£1,022	£511	841.5		
		£2,043	859.5		
CRC surgery	Segmental resection GP £6,514, LS £6,605 Subtotal colectomy IRA £7,879 Rectal excision £7,939 Proctocolectomy £7,977	Segmental resection GP £3,257, LS £3,302 Subtotal colectomy IRA 3,939 Rectal excision £3,969 Proctocolectomy £3,988	808.9		
		Segmental resection GP £13,028, LS £13,210 Subtotal colectomy IRA £15,757 Rectal excision £15,878 Proctocolectomy £15,953	924.7		
CRC chemo and radiotherapy	Colon Dukes' A, D £0, Dukes B £5,653, Dukes' C £12,900 Rectal Dukes' A £1,049, Dukes' B £4,206, Dukes' C £9,504, Dukes' D £0	Colon Dukes' A, D £0, Dukes B £2,826, Dukes' C £6,450 Rectal Dukes' A £525 Dukes' B £2,103, Dukes' C £4,752, Dukes' D £0	810.7		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
		Colon Dukes' A, D £0, Dukes B £11,306, Dukes' C £25,800 Rectal Dukes' A £2,099, Dukes' B £8,411, Dukes' C £19,008, Dukes' D £0	921.0		
CRC stoma care	GP £388 LS £214	GP £194 LS £107	845.9		
		GP £776 LS £429	850.6		
CRC follow up and surveillance	GP £230 LS £229	GP £115 LS £115	844.9		
		GP £460 LS £458	852.6		
CRC recurrence	GP £12,236 LS £12,333	GP £6,118 LS £6,166	803.6		
		GP £24,472 LS £24,666	935.2		
CRC palliative care	GP £9,665 LS £9,907	GP £4,833 LS £4,954	808.1		
		GP £19,331 LS £19,815	926.2		
EC related parameters					
Prophylactic H-BSO disutility	0	0.04 applied for 1 year (equal to EC disutility)	400.8		
EC disutility	0.04 applied for 1 year	0.07 applied for 1 year	782.2		
EC related costs		**Halved and doubled**			
Gynae screening	£473	£237	946.1		

Parameter	Base case value	Sensitivity analyses	INHB Strategy 5 (base case optimal strategy) vs no testing	Optimal strategy (if different from base case)	INHB vs. no testing at £20k/QALY optimal strategy in SA (QALYs)
Base case			**847.5**		
		£947	650.1		
Prophylactic H-BSO	£3,428	£1,714	950.2		
		£6,856	642.1		
EC related H-BSO	£4,005	£2,002	838.1		
		£8,009	866.2		
EC radiotherapy	£2,735	£1,367	831.2		
		£5,469	860.3		
EC chemotherapy	£324	£162	846.7		
		£647	849.0		
Disutility EC risk reduction (surveillance or H-BSO) declined	Test declined, risk reduction decline 0.11 LS+ve risk reduction declined 0.09	Test declined, risk reduction decline 0 LS+ve risk reduction declined 0	748.1		
		Test declined, risk reduction decline 0.22 LS+ve risk reduction declined 0.18	856.6		

Key: CRC, colorectal cancer; EC, endometrial cancer; GC, genetic counselling; GP, general population; GT, genetic testing; H-BSO, hysterectomy and bilateral salpingo-oophorectomy; HR, hazard ratio; INHB, incremental net health benefit; IRA, ileorectal anastomosis; LS, Lynch syndrome; QALY, quality-adjusted life year; SA, sensitivity analysis

5.3 Discussion

The analyses conducted suggest that screening for Lynch syndrome in colorectal cancer patients using tumour-based tests (in particular, IHC, *BRAF* and *MLH1* methylation) would be cost-effective at a threshold of £20,000 per QALY. Direct MMR mutation testing was predicted not to be cost-effective at any cost-effectiveness threshold as it was not on the cost-effectiveness frontier.

Subgroup analyses suggest that the use of a maximum age limit for testing does not significantly affect the cost-effectiveness of testing, although it does significantly affect the number of individuals affected. For this reason budget impact and total health benefit are smaller when a lower age limit is used. Using a minimum age limit for testing significantly worsens cost-effectiveness, but when a minimum age of 70 years is employed, testing is still predicted to be cost-effective at a threshold of £20,000 per QALY.

Scenario and sensitivity analyses indicate that there are some assumptions or parameters to which the cost-effectiveness results are sensitive. The most important costs in this respect are the cost of colonoscopy and the costs of MSI and IHC. Cost-effectiveness is also sensitive to the accuracy of tumour tests, the acceptance of genetic counselling and testing, and the number of relatives identified through cascade testing per proband. The effectiveness of surveillance colonoscopy and the lifetime risk of colorectal cancer for people with Lynch syndrome are also key determinants of cost-effectiveness.

The results are expected to be valid for the NHS (i.e., the results are expected to generalise to the NHS), partially since key costs were estimated from NHS sources, including NHS reference costs. Also, survival for colorectal cancer and endometrial were estimated from England or UK patients. The survival from cancers can vary even among high-income countries in Europe. The effectiveness of colonoscopic surveillance was estimated from a Finnish study, so it is possible that surveillance in the NHS may not have the same effectiveness. It is considered likely that the cancer risks estimated for individuals with Lynch syndrome are appropriate since they are estimated from a French population. The prevalence of Lynch syndrome in colorectal cancer patients was estimated from a US study, but there is no obvious reason to think the prevalence would differ markedly, since neither the UK nor the US has significant founder mutations.

6 Assessment of factors relevant to NHS and others

6.1 Current variability in implementation

The Royal College of Pathologists (RCPath) dataset for colorectal cancer indicates the value of assessing mismatch repair status:

> MMR status has prognostic significance, possible predictive significance and can help detect Lynch syndrome families. As such, a strong case can now be made for performing MMR immunohistochemistry in all cases of CRC. However, given the resource implications of implementing this, it is not considered a core data item for all colorectal cancers currently. We now consider MMR immunohistochemistry a core dataset item for patients under 50 years at time of diagnosis
>
> *—Page 5 of Loughrey et al. 2014*[47]

This has been in place for two years now and there have been efforts from the charity Bowel Cancer UK, through Freedom of Information (FOI) requests, to document compliance with this core item (MMR immunohistochemistry for patients under 50).[175]

Of the 130 hospitals in England which responded (83% of the 159 contacted), 90 (69%) indicate that all patients diagnosed with bowel cancer under age 50 are tested with MSI or IHC, but only 49 of these (54%) conduct them as reflex tests (i.e., automatically, without referral).

Hospitals which have not implemented routine testing cite finances and practicalities as principal barriers to implementation.[175]

This suggests that there is current variability in the level of implementation (some hospitals implement testing for colorectal cancer patients under 50, while others do not) and the pathway (reflex testing versus referral).

6.2 Use and impact of age limits

Age limits for testing and surveillance have been suggested for Lynch syndrome by numerous expert groups,[19, 30, 47, 118] usually with the intention of balancing clinical benefits against resource implications and clinical risks.

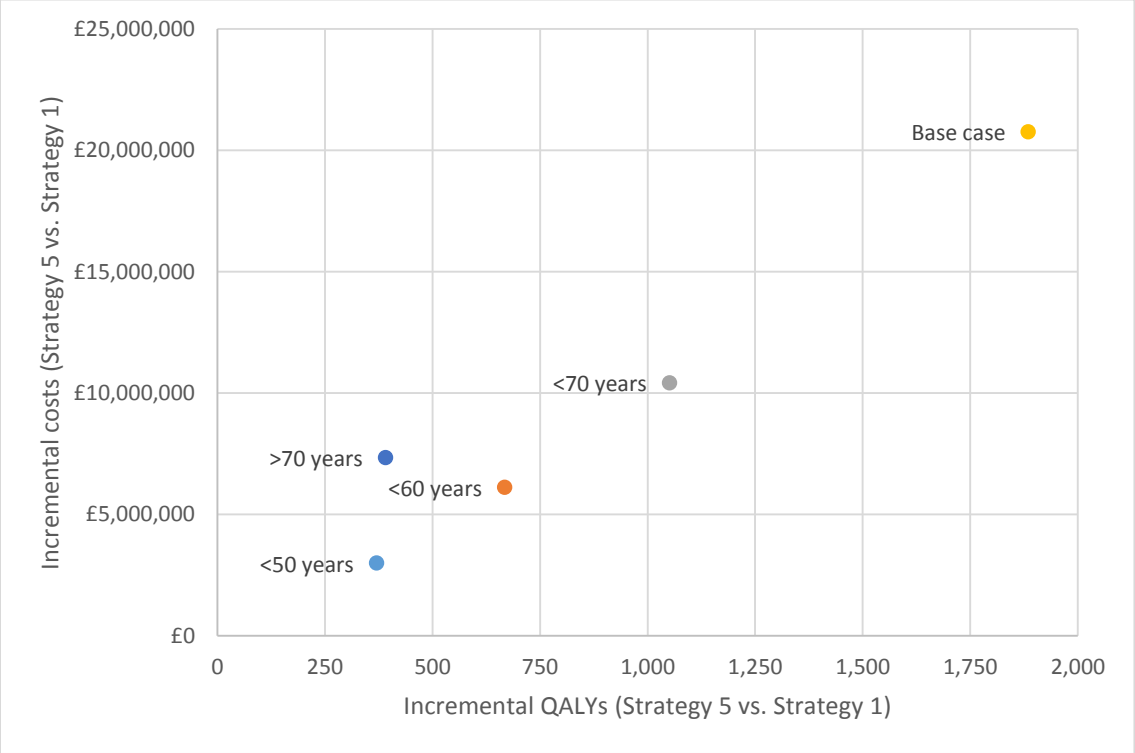
The Equality Act 2010 applies to NICE, which is required to have due regard to the need to eliminate discrimination.[176] Age is a protected characteristic in this context.

In the context of screening for Lynch syndrome in colorectal cancer patients, age is a proxy for the pre-test probability of having Lynch syndrome[85] and therefore directly related to the potential for an individual to benefit from screening. While there are other observable characteristics which could also be used to identify a higher risk group (such as tumour morphology associated with MMR deficiency[47]), assessing these would increase the complexity of the service pathway, and could result in reduced implementation.

In accordance with the NICE Scope, subgroup analyses were conducted on a number of age groups. As in the base case, Strategy 5 is the cost-effective strategy in all subgroup analyses (except when an age limit of 50 is used, in which it is a close second; assuming a cost-effectiveness threshold of £20,000 per QALY). *Figure 60* shows the incremental costs

and QALYs for Strategy 5 versus Strategy 1 in the different analyses. The greatest incremental net health benefit (at a willingness-to-pay of £20,000 per QALY) is obtained in the base case (848 QALYs). This is as a result of having the largest population, and the ratio of incremental costs to QALYs being relatively stable as a maximum age limit is progressively lifted.

Figure 60: Incremental costs and QALYs for Strategy 5 versus Strategy 1 in the base case and across different subgroup analyses



Imposing a minimum age limit of 70 years has a detrimental impact on cost-effectiveness, and has not been recommended by any organisations. However, using base case assumptions and parameter values, testing using Strategy 5 is still cost-effective at a cost-effectiveness threshold of £20,000 per QALY (ICER £18,774 per QALY).

The decision modelling has assumed that there is no direct cost to imposing an age threshold (which is probably a reasonable assumption) but also that the imposition of an age threshold does not result in reduced compliance with reflex testing.

7 Discussion

7.1 Review of test accuracy evidence

7.1.1 Findings in relation to previous studies

Ten studies met the test accuracy review inclusion criteria. One of the included studies had two distinct samples (a population-based sample and a high-risk sample). Therefore, there were 10 included studies with 11 included populations/samples. IHC was conducted in all 10 studies (11 samples). However, not all studies provided sufficient data to be included in IHC analyses. Indeed, in two study samples (the high-risk sample in Poynter, 2008 and Mueller 2009), insufficient data were provided for any of the IHC analyses.[31, 56]

A variety of study samples were included as follows:

- Four single-gate studies with population-based samples, including an unselected CRC sample (Poynter, 2008)[31] and three age-limited populations (Barnetson 2006, Limburg, 2011, Southey 2005)[52-54]
- Five single-gate studies based on high-risk populations (Caldes 2004, Mueller 2009, Overbeek 2007, Poynter 2008, Shia 2005)[31, 55-58]
- Two studies that were a variation on a two-gate study design (Hendriks 2003, Okkels 2012)[59, 60] where participants with positive reference standard results were recruited but no reference standard negatives were recruited, and termed reference standard positive studies for this report

Overall, there were no concerns about whether or not the included participants matched the review question. The reference standard was assessed as likely to correctly classify the target condition and there were no concerns that the target condition, as defined by the reference standard, did not match the review question. However, all studies were rated as unclear with regards to whether the conduct and interpretation of the test could have introduced bias. It was also unclear, for all studies, whether the flow of participants through the study could have introduced bias.

Only four studies were rated as having a low risk of bias due to patient selection (Barnetson, 2006; Limburg, 2011; Southey, 2005; Okkels 2012).[52-54, 60] However, none of the studies were rated as having a high risk of bias due to patient selection, even though the index tests are highly susceptible to spectrum effects in populations that have been selected due to clinical characteristics. This is because it was decided prior to conducting the review that only sensitivity estimates would be made for studies recruiting high-risk samples. This decision was based on the fact that a previous review (Palomaki, 2009) did not find significant bias in estimates of sensitivity due to recruitment of high-risk samples.[39]

7.1.1.1 Evidence for MSI

With the exception of the studies by Limburg (2011) and Okkels (2012), all studies assessed MSI.[53, 60]

7.1.1.1.1 Sensitivity results for MSI

Across all nine study samples (population-based and high-risk), sensitivity ranged from 66.7% (95% CI 47.2, 82.7) to 100.0% (95% CI 93.9, 100.0) when MSI-L was considered to be a negative result. A previous review by Palomaki (2009) pooled data from 11 studies (one age-limited sample and ten high-risk samples) in a random effects model and reported the sensitivity of MSI, according to which gene a mutation was found in, to be 85% (95% CI 75, 92) for identifying MLH1 mutations and 85% (95% CI 73, 93) for MSH2 mutations.[39] Data were pooled from five studies to produce a sensitivity of MSI of 69% (95% CI 46, 85) for identifying MSH6 mutations. In all of these analyses, MSI-L was considered to be a negative index test result. It is difficult to compare these results with the ones produced for this review, primarily because different inclusion criteria were used, in particular for the reference standard, in this review and the review by Palomaki (2009).[39] In addition, data were not pooled in this review, and not split according to gene. Nevertheless, the sensitivities and confidence intervals provided in Palomaki (2009) were similar to those in this review.[39] Similarly, a review by Bonis (2007) provides an overall sensitivity for MSI-H versus MSS from 16 studies with a relatively wide range of 56% to 100%. Again, although results appear similar to those from this review, due to differences in the inclusion criteria used in the review by Bonis (2007) and this review, comparison may not be particularly meaningful. For example, in Bonis (2007) any genetic testing was included as the reference standard so a wider range of studies were included.[18]

In this review of test accuracy, it was noted that sensitivity increased if MSI-L was included as a positive index test result. Indeed, across the nine study samples in this review, the lower end of the range for sensitivity increased to 79.4% (95% CI 62.1, 91.3) with the higher end remained at 100%. The review by Bonis (2007) reports seven studies where MSI-L was considered to be a positive index test result and also found increased sensitivity estimates, with pooled data producing a summary estimate for sensitivity of 94% (95% CI 86, 97).[18] Indeed, when MSI-L tumour results were excluded from analyses, summary sensitivity was reported as 80% (95% CI 63, 90), because the number of positive results reduced. As previously mentioned, it is unsurprising that including MSI-L as index test positive results increases sensitivity because doing this essentially decreases the threshold for a positive test result.

Another review, by Snowsill (2014),[4] also report sensitivity data for MSI, ranging from 88% to 100%, when MSI-L was considered to be a positive index test result. Again, although these estimates are similar to those reported in this review, comparison may not be particularly useful due to the application of different inclusion criteria; the review by Snowsill (2014) included nine high-risk or age-limited studies (studies were included if the participants were at risk of Lynch syndrome due to being <50 years at diagnosis, or due to clinical criteria or family history indicators), studies were also included where not all patients received the reference standard, and looser criteria were applied to the reference standard than those applied for this review. It is notable, however, that despite these differences, results were similar. This may be, in part, due to a lack of data identified in this review from unselected CRC populations.

7.1.1.1.2 Specificity results for MSI

Three population-based study samples provided data where MSI-L was considered to be a negative index test result (Poynter, 2008; Barnetson, 2006; Southey, 2005).[31, 52, 54] Across

these three samples, specificity ranged from 61.1% (95% CI 57.0, 65.1) in Poynter (2008) to 92.5% (95% CI 89.1, 95.2) in Barnetson (2006). It should be noted that Barnetson (2006) was based on an age-limited sample whereas Poynter (2008) was based on an unselected CRC population.[31, 52] Six studies that provided data where MSI-L was considered to be a negative result were pooled in the review by Palomaki (2009) with summary specificity given as 90.2% (95% CI 87.7, 92.7).[39] This result is closer to the specificity reported by Barnetson (2006) in this review and this may be, in part, because Palomaki (2009) included only high-risk or age limited populations. The review by Bonis (2007) reports an overall specificity from 16 included studies for MSI-H vs MSS to be between 17% and 93%, indicating substantial heterogeneity. [18] Again, it is difficult to compare the results from these previous reviews with this review, due to key differences in the inclusion criteria applied.

In this review, and as expected, specificity decreased when MSI-L was considered to be a positive index test result. The lower end of the range decreased to 29.5% (95% CI 25.8, 33.4) in Poynter (2008) and the upper end of the range to 84.5% (95% CI 80.0, 88.2) in Barnetson (2006).[31, 52] Indeed, pooled data from seven studies included in Bonis (2007), estimated specificity at 83% (95% CI 77, 88) when MSI-L was considered to be a positive index test result, whereas specificity was estimated as 88% (95% CI 83, 91) when MSI-L tumours were excluded. However, the authors note substantial statistical heterogeneity in both analyses.[18] Snowsill (2014) report a specificity range of 68 to 84% when MSI-L was considered to be a positive test result, the upper end of which is comparable to this review.[4] However, the review by Snowsill (2014) was based only upon high-risk or age-limited studies, and so does not include the population-based sample from Poynter (2008).[31]

7.1.1.2 Evidence for IHC

Seven study samples (Barnetson, 2006; Limburg, 2011; Southey, 2005; Caldes, 2004; Overbeek, 2007; Shia, 2005; Hendriks 2003) provided data to assess the accuracy of an overall IHC result at identifying a positive reference standard result (i.e., whether a positive IHC result, regardless of which protein this applies to, identifies a positive reference standard result).[52-55, 57-59] All seven of these studies assessed MLH1, MSH2 and MSH6 proteins.[52-55, 57-59] The studies by Southey (2005) and Overbeek (2007) also assessed PMS2.[54, 57] Five study samples (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) split IHC data according to the particular protein assessed (MLH1, MSH2, MSH6 or PMS2).[31, 52, 54, 59, 60]

7.1.1.2.1 Overall sensitivity and specificity results for IHC

Sensitivity estimates ranged from 80.8% (95% CI 60.6, 93.4) to 100.0% (95% CI 81.5, 100.0 for IHC overall.[54, 58] The study by Southey (2005) included an assessment of PMS2 as well as MLH1, MSH2 and MSH6, which may account for the higher sensitivity estimate in this study.[54] Nevertheless, all sensitivity estimates were >80%. Specificity was estimated as 91.9% (95% CI 86.3, 95.7) for Limburg (2011) and 80.5% (95% CI 65.1, 91.2) for Southey (2005).[53, 54]

Other reviews have produced similar results, but again, it must be noted that these reviews used different inclusion criteria to this one. The review by Bonis (2007) produced a summary sensitivity of 74% (95% CI 54, 87) and specificity of 77% (95% CI 61, 88) for IHC overall, based upon six studies considered to be good or fair quality.[18] Similar results were reported by Palomaki (2009) based on pooled data from three studies; overall sensitivity was

estimated as 77% (95% CI 69, 84) and specificity as 88.8% (95% CI 67.6, 94.8), although statistical heterogeneity was noted.[39] Similarly, without pooling data, Snowsill (2014) report a sensitivity range of 73.3% to 100.0% and a very wide ranging specificity of 12.5% to 100%.[4] Indeed, Snowsill (2014) discuss how specificity appears to be the greatest concern with IHC, as the high number of FPs means that individuals may be told they have Lynch syndrome when they do not. However, in the two studies included in this review, specificity of IHC was >80%. This difference is likely because the specificity data from the review by Snowsill (2014) was based on high-risk as well as age-limited studies, whereas to mitigate spectrum effects, the specificity data from this review was based only upon two studies that recruited age-limited populations.

7.1.1.2.2 Sensitivity and specificity for IHC by individual protein

In five study samples an assessment of IHC was made for at least one individual protein (the population-based sample in Poynter, 2008; Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012).[31, 52, 54, 59, 60] With regards to estimating sensitivity, three of these studies provided data relevant to whether loss of expression in MLH1 was an accurate test result for assessing a pathogenic mutation in MLH1(Barnetson, 2006; Southey, 2005; Hendriks, 2003)sensitivities ranged from 50.0% (95% CI 26.0, 74.0) to 100.0% (95% CI 73.5, 100.0).[52, 54, 59] The results for MSH2 were even more variable; the same three studies provided data and sensitivities ranged from 22.2% (95% CI 6.4, 47.6) to 81.8% (95% CI 48.2, 97.7). Four studies provided data for MSH6 (Barnetson, 2006; Southey, 2005; Hendriks, 2003; Okkels, 2012) and again, there was substantial variability; sensitivities ranged from 44.4% (95% CI 21.5, 69.2) to 75.0% (95% CI 19.4, 99.4).[52, 54, 59, 60] Only the study by Southey (2005) provided IHC data for PMS2, providing a sensitivity estimate of 55.6% (95% CI 30.8, 78.5).[54]

The results for specificity displayed less variability. Three population-based studies (Barnetson, 2006; Southey, 2005; Poynter, 2008) provided data enabling the estimation of specificity of loss of expression in MLH1 for identifying pathogenic mutations in MLH1.[31, 52, 54] These specificities ranged from 70.6% (95% CI 66.8, 74.2) to 96.0% (95% CI 93.1, 97.9). Two population-based studies (Barnetson, 2006; Southey, 2005) provided data for MSH2 and MSH6 with all specificities being >92%.[52, 54] Only the study by Southey (2005) provided IHC data for PMS2, providing a specificity estimate of 87.8 (95% CI 73.8, 95.9).[54]

The review by Palomaki (2009) investigated whether overall IHC results indicated gene-specific pathogenic mutations.[39] However, none of the previous systematic reviews identified through database searching provide an evaluation of whether loss of expression in a particular protein indicates a pathogenic mutation in that protein.

7.1.2 Strengths

The strengths of this systematic review are that it was conducted by an independent, experienced research team using the latest evidence and working to a pre-specified protocol (PROSPERO CRD42016033879) which follows a robust methodology.

The search strategy was devised by an information specialist and did not restrict by study design and also included both forward and backward citation chasing. The studies were independently screened by two reviewers, with data extraction and quality appraisal performed by one reviewer and checked by a second.

7.1.3 Weaknesses

The relatively low prevalence of Lynch syndrome means that comprehensive MMR gene testing among all CRC patients in the general population would be expensive. Therefore, as mentioned previously, five of the ten studies included in this review were single-gate studies based on high-risk populations (Caldes 2004, Mueller 2009, Overbeek 2007, Poynter 2008, Shia 2005). There is some concern that increased presence of MMR mutation carriers in a population may change the apparent sensitivity and specificity of the index tests.[31, 55-58 52] However, we did not use these studies to estimate specificity, and we did not find great differences between the studies with regards to sensitivity, although, this may be because the three population-based studies with overall IHC data were based on age-limited populations and, therefore, may also be subject to spectrum bias.[52-54] Palomaki (2009) also suggest that spectrum bias does not seem to have been an issue for estimating sensitivity. For example, when the population-based results for those younger than 55 years (80% for MLH1 and 82% for MSH2) are compared with the other four studies that were based on strong family histories regardless of age (82% for MLH1 and 88% for MSH2), the results are remarkably similar. Again, this may be due to some amount of spectrum bias as occurring for the age-limited population. The inclusion of high-risk studies in this review could, therefore, have introduced spectrum bias in sensitivity estimates, although this does not appear to be the case. The inclusion of high-risk studies in this review does mean that there are several studies from which sensitivity estimates could be made despite the low number of population-based studies identified (only one unselected CRC sample and three age-limited samples). However, this low number of population-based studies has resulted in limited specificity data, as well as limited data on other outcomes (predictive values and likelihood ratios).

Although not a weakness of the review methods per se, significant methodological and clinical heterogeneity was noted across the included studies and this impacts upon both the type of synthesis that could be conducted in this review and the way in which the findings should be considered. In particular, when considering the results, it is important to note that the reference standard differed between studies, as did the index tests. With regard to the reference standard there were differences in the testing methods used (including sequencing methods and genes tested, techniques used to test for large genomic alterations and deletions, genes tested for large genomic alterations and deletions, and whether unclassified variants were investigated).

For MSI, microdissection techniques were not always reported, the panel of markers varied, as did thresholds and categorisation of MSI. Indeed, none of the population-based studies in this review assessed the same panel of MSI markers. Issues with heterogeneity have also been reported in other similar reviews. For example, Bonis (2007) report they could not explain heterogeneity among estimates in sensitivity and specificity based on the overall study quality, the comprehensiveness of the genetic testing, the presence of microdissection, the use of NCI-recommended marker sets, or whether the study used a transparent sample selection process.[18] As with this review, Palomaki (2009) found weaknesses in the studies included in the review, as well as clinical and methodological heterogeneity between studies. [39] For example, none of the studies included in their review explicitly reported laser microdissection, which has been reported to be the optimal method for sample preparation.[39] In addition, none of the studies reported a minimum proportion of tumour cells. Roughly half of the studies, however, relied solely on the 1998 NCI

recommended panel that includes only two mononucleotide markers, whereas the remaining studies utilized three or more mononucleotide markers. When the results were stratified by this quality measure, the clinical sensitivities of studies using three or more mono-nucleotide markers were higher than those using two mononucleotide markers.[39] This provides some evidence to suggest that additional mononucleotide markers (up to five) should be included in an MSI panel for clinical testing. It was not possible to confirm this by running similar analyses in this review; in order to focus on the best reference standard testing methods, fewer studies were included, so insufficient homogenous data were available to perform similar analyses. Indeed, the clinical and methodological heterogeneity in the included studies precluded any statistical pooling of data. Instead results were discussed in a narrative synthesis.

It should also be noted that the review of test accuracy included only studies published in the English language and only published literature. In order to enable to full evaluation of the methodological quality of included studies, those studies published only as abstracts were also excluded.

It is possible that the estimates derived from the included studies are subject to some mount of publication bias. There was insufficient data from included studies to perform a statistical assessment of publication bias.

The bibliographic searches were conducted in February 2016 and therefore it is possible that relevant studies have been published or indexed subsequently but have not been identified. There were also no attempts to review “grey literature” such as technical reports which would not have been peer reviewed.

7.1.4 Areas of uncertainty

The greatest area of uncertainty is in the generalisability of the results to the general CRC population from high-risk and age-limited studies. As discussed above, only one study sample was identified that was based on an unselected CRC population (Poynter, 2008).[31] This is unsurprising given that large population-based studies where all participants receive both the index test and the reference standard would be costly. Performing the reference standard on a random sample of index test negatives would be methodologically acceptable, and would somewhat decrease the costs of performing a population-based study where all participants receive all tests.

Another area of uncertainty is the categorisation of unclassified variants; three of the four population-based studies (Poynter, 2008; Limburg, 2011; Barnetson, 2006) report on unclassified variants (i.e., mutations where the association with Lynch syndrome is unclear).[31, 52, 53] This can complicate the assessment of MSI in particular, as the variant has uncertain pathogenicity and may occur in cases with either MSI-H or MSS tumours. In this review, in primary analyses, unclassified variants have been counted as reference standard negative results. However, secondary analyses were also conducted, as appropriate, where unclassified variants were considered to be reference standard positives.

7.1.4.1 Unclassified variants – MSI

Two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct secondary analyses where unclassified variants were categorised as positive reference standard results.[55, 59] Caldes (2004) was based on a high-risk population and Hendriks (2003) is a reference standard positive study, so only sensitivity estimates were made. When MSI-L was

considered to be a negative index test result, Caldes (2004) reported sensitivity as 81.6% (95% 65.7, 92.3) and Hendriks (2003) reported sensitivity as 84.8% (95% CI 69.0, 93.3).[55, 59] These results were similar to those obtained when unclassified variants were considered to be negative (79.4% (95% CI 62.1, 91.3) for Caldes (2004) and 88.8% (95% CI 68.8 to 97.5) for Hendriks (2003)).[55, 59]

When MSI-L was considered to be a positive index test result, only one study (Hendriks, 2003) provided sufficient data to conduct a secondary analysis of the sensitivity estimate.[59] In this study, sensitivity was 93.9% (95% CI 80.3, 98.3) which was similar to when unclassified variants were considered to be negative (92.0%; 95% CI 74.0 to 99.0).

7.1.4.2 Unclassified variants – IHC

As for MSI, only two studies (Caldes, 2004; Hendriks, 2003) provided sufficient data to conduct these secondary analyses.[55, 59] Again, because Caldes (2004) was based on a high-risk population and Hendriks (2003) is a reference standard positive study, only sensitivity estimates were made (75.0%, 95% CI 57.8, 87.9 for Caldes, 2004; 88.6%, 95% CI 76.0, 95.0 for Hendriks, 2003).[55, 59] For Caldes (2004) this represents quite a reduction in sensitivity compared to when unclassified variants were considered to be index test negatives (96.4%; 95% CI 81.7, 99.9).[55]

7.2 Review of end-to-end studies

7.2.1 Findings in relation to previous studies

No studies were identified which met the inclusion criteria of the review.

The review by Bonis et al. (2007)[18] also attempted to identify end-to-end studies (their Key Question 1) but identified none.

7.2.2 Strengths

The review was conducted by an experienced researcher and the searches employed were very sensitive as no study design filters were employed. A second reviewer parallel screened a random sample of bibliographic records and excellent agreement was achieved. Although no studies were eventually included, the prospective protocol pre-specified the quality appraisal strategy.

7.2.3 Weaknesses

The review focused on published literature indexed by bibliographic databases, meaning that grey literature was not identified. The searches were conducted in February 2016, so it is possible that studies have been published and indexed subsequently which have not been identified.

7.2.4 Areas of uncertainty

Since no studies were identified for inclusion, there is no high-quality published evidence that screening for Lynch syndrome in colorectal cancer patients using MSI or IHC improves health outcomes.

Given that it is widely believed that such screening would improve health outcomes it is unlikely that high-quality evidence will be generated in the future, e.g., in the form of a randomised controlled trial. What evidence may be produced in the future will likely be

observational, and though statistical methods may be used to remove certain biases, it is likely that there will be certain biases which cannot be adjusted for, and that statistical power will be low compared to what could be produced by a randomised controlled trial.

7.3 Review of existing cost-effectiveness evidence

7.3.1 Findings in relation to previous studies

The findings of the review are that while no studies fully answered the decision problem specified by the NICE Scope, studies generally found that some testing strategy was cost-effective (according to the relevant perspective) and that tumour-based testing usually improved cost-effectiveness versus direct genetic testing. The effectiveness of colonoscopy, the number of relatives receiving cascade genetic testing and the prevalence of Lynch syndrome in the population were generally identified as key parameters to which results were sensitive.

These results are similar to the previous review by the authors.[4]

Other reviews of cost-effectiveness have been published. Grosse (2015)[72] similarly found that studies usually produced one or more cost-effective strategy, but that these sometimes used age limits or clinical criteria. The number of first-degree relatives identified for cascade genetic testing and the cost of gene sequencing were found to be significant, as were the frequency and cost of surveillance colonoscopy, and the inclusion of extracolonic surveillance.

Ladabaum et al. (2015)[3] also review the health economic literature for tumour testing, and conclude that it is estimated to be cost-effective, especially if cascade genetic testing is employed.

7.3.2 Strengths

This review was conducted by an independent, experienced research team using the latest evidence and working to a pre-specified protocol (PROSPERO CRD42016033879). Screening was conducted independently by two reviewers.

7.3.3 Weaknesses

Data extraction and quality appraisal were conducted by one reviewer. This has the potential to lead to inconsistencies and misinterpretation between the published studies and the review reporting.

As with all reviews of published literature, this review is open to publication bias and so some information may have been missed. The searches were conducted in February 2016, so it is possible that studies have been published and indexed subsequently which have not been identified.

More recent quality appraisal tools are available. Drummond and Jefferson (1996)[83] was used for consistency with the previous review, but the Philips or Evers checklists may now be more appropriate.

7.3.4 Areas of uncertainty

As the reporting was of mixed quality and some studies only reported in abstract, the data extracted from the studies and conclusions drawn are likely to reflect this.

The results of the review highlighted the need for a cost-effectiveness analysis specific to the decision problem. No individual studies were able to address this and the results could not be adequately synthesised, given the combination of different strategies, modelling techniques, and perspectives of the studies.

Though the included studies indicated that a strategy of diagnostic testing for Lynch syndrome is likely to be cost-effective for each of the described contexts, conclusions could not be drawn with regards which strategies would be most cost-effective, particularly from the perspective of the NHS.

7.4 Independent economic assessment

7.4.1 Findings in relation to previous studies

The base case estimates from the independent economic assessment suggest that testing for Lynch syndrome in colorectal cancer patients would be cost-effective (compared to not testing) at a cost-effectiveness threshold of £20,000 per QALY, except in the case where patients are offered comprehensive genetic testing for Lynch syndrome without any tests being conducted on the tumour. In this case, testing is estimated to be cost-effective compared to no testing at a threshold of £30,000 per QALY. This universal testing strategy is, however, not predicted to be cost-effective in a fully incremental analysis (in which testing with MMR immunohistochemistry, *BRAF* V600E testing and *MLH1* hypermethylation testing is cost-effective). Indeed, it is estimated that the universal genetic testing strategy would be more expensive and less effective than another strategy.

These findings are similar to those in the previous PenTAG economic evaluation[4] (in particular the scenario analysis where the age limit for testing is raised to 70 years) even though in the base case of this assessment there is no age limit and there have been a number of changes to the analysis (e.g., new diagnostic strategies, new parameter estimates for diagnostic accuracy, inclusion of aspirin chemoprevention and gynaecological surveillance).

The current analysis suggests that IHC-based testing would be more cost-effective than MSI-based testing, whereas the previous PenTAG analysis suggested the opposite. This is partially because the sensitivity of IHC modelled now is higher than before, and the specificity of MSI lower. There is no compelling evidence that either test has superior diagnostic performance, and costs for both tests can vary between settings (e.g., in some hospitals a pathologist will be able to perform IHC but there will be no facility for MSI so samples will need to be sent elsewhere at a cost). *BRAF* and/or *MLH1* hypermethylation testing are only conducted on patients with abnormal IHC results where the MLH1 protein staining is abnormal, whereas these tests are conducted for all patients with MSI (so all else being equal there would be a reduction in downstream costs if IHC were used). IHC is also required in a small minority of cases to assist in the interpretation of MMR mutation testing, and therefore some individuals receiving MSI testing initially may later receive IHC as well.

There are no other UK-based cost–utility analyses for further comparison, but the results of this study are also comparable to a number of studies from elsewhere. For example, Mvundura et al.[77] (a US study) found that tumour based testing (using IHC and *BRAF* testing) was cost-effective (ICER $22,552 per life-year), while direct genetic testing was not cost-effective (ICER > $142,289 per life-year compared to no testing and ICER $737,025 per life-year in a fully incremental analysis). Wang et al.[79] (another US study) found that tumour

based testing may be cost-effective (ICER $59,719 per QALY) but that direct genetic testing would not be cost-effective (ICER $271,219 per QALY).

7.4.2 Strengths

The economic evaluation was conducted by an independent academic group with experience in reviewing and modelling in this disease area. The population, interventions, comparators and outcomes are based on the NICE Scope.

The model was built by extending an existing peer-review model which had previously been quality assured and peer reviewed. The extensions mean the model now more closely matches likely clinical practice.

The model methodology (individual patient simulation) allows for detailed modelling, including multiple cancers, different colorectal cancer stages, evolving strategies for gynaecological risk reduction, event probabilities which can be dependent on the time since previous events. This means there was no need to make assumptions of constant hazards of events (i.e., exponential survival distributions).

The evidence for diagnostic test accuracy parameters was identified through systematic review. Evidence for key model parameters was identified by reviewing the published literature and critically appraising alternative sources.

Scenario and sensitivity analyses were conducted to identify key sources of decision uncertainty.

The model was quality assured by developers testing each other's components.

7.4.3 Weaknesses

Weaknesses of the systematic review of test accuracy are carried through to the economic assessment, since the cost-effectiveness of testing strategies are sensitive to the test accuracy parameter values.

The model base case uses sensitivity and specificity estimates for MSI and IHC which are from a meta-analysis of studies identified in the systematic review of test accuracy, even though for a number of reasons meta-analysis was not felt to be appropriate for the review.

The model assumes that diagnosis of Lynch syndrome in the probands and relatives occurs at the model start, i.e., that there are no delays to testing, and that surveillance does not commence before testing is completed.

To achieve convergence in model estimates it was estimate to simulate 240,000 individual patients. A probabilistic sensitivity analysis (PSA) was not conducted due to the computational requirements of such an analysis, and therefore decision uncertainty has only been explored using scenario analyses and sensitivity analyses with limited numbers of parameters varied simultaneously.

The model does not include ovarian cancer, small bowel cancer, gastric cancer, or other cancers associated with Lynch syndrome. Of these, ovarian cancer would be most likely to affect cost-effectiveness, since the model already includes the costs of risk-reducing interventions for ovarian cancer (these are the same as those for endometrial cancer) but none of the benefits.

7.4.4 Areas of uncertainty

There is significant uncertainty as to the true risk of colorectal cancer for individuals with Lynch syndrome not receiving colorectal surveillance, and as to the true effectiveness of colorectal surveillance in reducing the risk of colorectal cancer. The cost-effectiveness of testing strategies are sensitive to both of these parameters. Neither parameter is likely to be directly investigated in an experimental context (e.g., a randomised controlled trial) for ethical reasons. The parameter values for each were chosen with care following a review of the literature, and are more conservative than estimates made in other economic evaluations and systematic reviews. For example, a recent technical review estimated that CRC incidence would be reduced more heavily by colorectal surveillance than we have estimated,[3] and most reports quote a higher range of lifetime colorectal cancer risks than we have modelled. Univariate sensitivity analyses have been conducted on these parameters, but two-way sensitivity analysis was not conducted.

There remains substantial uncertainty in the estimates of test accuracy measures for MSI and IHC, since there are few studies investigating test accuracy which are not at high risk of bias (e.g., due to applying the reference standard only when the index test is positive or some family history criteria are met). If the true sensitivity and specificity of MSI are as low as explored in a sensitivity analysis, it would not be cost-effective (versus not testing) to screen using MSI-based strategies (at a cost-effectiveness threshold of £20,000 per QALY).

It appears that gynaecological surveillance and aspirin do not have a significant impact on cost-effectiveness, and therefore the uncertainty in the extent to which they are offered to patients is unlikely to translate into decision uncertainty.

8 Conclusions

There is evidence that microsatellite instability (MSI) testing and mismatch repair (MMR) immunohistochemistry (IHC) are effective tests to identify colorectal cancer patients who may have Lynch syndrome. This evidence comes from ten published studies which were judged to not be at high risk of bias. Some studies did not include patients known not to have Lynch syndrome, and could therefore not provide estimates of specificity for the tests. The reference standard (comprehensive genetic testing) was variable across the studies and cannot be considered a gold standard, particularly due to the number of variants of uncertain significance (VUS) identified. A number of studies used clinical criteria (or other means) to select from a high-risk population for testing, which puts their results at risk of spectrum bias.

Due to the limited number of population-based studies and the heterogeneity of test accuracy estimates from these studies, it was judged that quantitative synthesis (meta-analysis) should not be conducted as part of the systematic review.

It is primarily cost considerations which prohibit a large-scale high quality assessment of the diagnostic test accuracy of MSI and IHC in colorectal cancer patients. Conducting both index tests and the reference standard would cost over £1,000 per patient.

No end-to-end studies were identified which directly investigated the impact of screening for Lynch syndrome in colorectal cancer patients on long-term outcomes such as survival or cancer incidence.

It is unlikely that any high quality end-to-end studies will be conducted (e.g., randomised or cluster randomised controlled trials), due to ethical considerations; it is believed, and supported by modelling, that identifying Lynch syndrome-causing mutations and offering risk-reducing interventions leads to clinical benefits for patients, so there would not be clinical equipoise.

The majority of health economic evaluations of screening for Lynch syndrome in colorectal cancer patients have suggested that it may be cost-effective to screen using MSI or IHC. These studies, however, may not reliably generalise, and therefore a new economic evaluation was conducted for this report.

The results of the economic evaluation suggest that it is likely to be cost-effective (at a threshold of £20,000 per QALY) to screen colorectal cancer patients using IHC, *BRAF* V600E testing and *MLH1* methylation testing. This is estimated to extend the life expectancy of patients with Lynch syndrome by around one year and the life expectancy of their relatives with Lynch syndrome-causing mutations by two years. There are estimated to be 956 such patients and 2,524 relatives per year. The diagnosis costs are estimated to be around £10.5 million per year (versus an estimated £1.9 million to be conducted to direct 5-FU chemotherapy response). It is estimated that additional QALYs would be gained at an incremental cost of £11,000 per QALY.

The cost-effectiveness of screening for Lynch syndrome is estimated to be most sensitive to:

- The effectiveness of surveillance colonoscopy in reducing CRC incidence;
- The diagnostic test accuracy of MSI and IHC;

- The proportion of patients declining genetic counselling and/or genetic testing after tumour testing;
- The lifetime risk of colorectal cancer for individuals with Lynch syndrome-causing mutations;
- The number of relatives identified through cascade testing;
- The cost of colonoscopy.

Of these, the cost of colonoscopy is most precisely known (on average; the cost does vary between Trusts), although the frequency of colonoscopy should be considered – the model assumes biennial colonoscopy, but costs would be significantly increased if annual colonoscopy were implemented.

The number of relatives identified through cascade testing and the proportion of patients declining genetic counselling and/or genetic testing after tumour testing can be estimated retrospectively from clinical records, or monitored prospectively, but the values employed in sensitivity analyses are likely worst case scenarios.

The sensitivity and specificity of MSI and IHC could not be directly monitored by introducing the tests into clinical practice (although the positive predictive value could be estimated). As explained above, a comparative diagnostic test accuracy study would be relatively expensive to conduct. When sensitivity and specificity of IHC, *BRAF* and *MLH1* methylation testing are all estimated from their respective lower 95% confidence limits it is still predicted that screening is cost-effective with a cost-effectiveness threshold of £20,000 per QALY.

The effectiveness of surveillance colonoscopy and the lifetime risk of colorectal cancer for individuals with Lynch syndrome-causing mutations (in the absence of risk-reducing interventions) are subject to significant uncertainty, for the reason that they cannot be simply observed without bias.

The effectiveness of surveillance colonoscopy cannot be examined in a randomised controlled trial for ethical reasons, and is therefore generally only estimated by comparing the risks observed in individuals undergoing screening to the risks in those diagnosed with Lynch syndrome and declining surveillance or those not diagnosed with Lynch syndrome but later established to have Lynch syndrome. Neither of these comparisons is between like groups.

The lifetime risk of colorectal cancer for individuals with Lynch syndrome-causing mutations but without risk-reducing interventions can generally only be estimated by studying the cancer incidence prior to diagnosis and commencement of risk-reducing interventions, and also correcting for ascertainment bias. Estimates are therefore of limited precision even from large studies.

When either of these parameters is modelled at its extreme value, the cost-effectiveness of screening is marginal at a cost-effectiveness threshold of £20,000 per QALY (ICERs ~£19,000 per QALY). The extreme value for the effectiveness of colonoscopy (zero effectiveness) may not be considered clinically likely, but the extreme value for the lifetime colorectal cancer risk is a lower 95% confidence limit. The base case estimates for these parameters are from different populations in different studies, but if a study were designed to estimate both parameters there would be significant correlation in the parameter estimates, since a low observed cancer risk in those undergoing surveillance could result from

surveillance being highly effective or the underlying risk already being low. This would mean that the precision in estimated cost-effectiveness would not be increased as much as if the parameters were not correlated.

One way to partially ameliorate this issue might be to delay colorectal surveillance for individuals with *MSH6* or *PMS2* mutations to 10–20 years later than for individuals with *MLH1* or *MSH2* mutations.[2, 5] It is considered that this could save 5–10 colonoscopies per individual with a minimal increase in the number of colorectal cancers.

It is also possible that other developments in the management of individuals with Lynch syndrome-causing mutations, or the management of colorectal or endometrial cancer, will lead to shifts in the cost-effectiveness of screening. For example, a vaccine targeting frameshift peptides (FSPs) which are produced by MSI-H cancer cells is considered to be a promising avenue for treating MSI-H cancer and for cancer prevention in individuals with Lynch syndrome.[177]

We have also assumed that treatment for colorectal cancer does not include monoclonal antibody chemotherapy, which means the costs associated with colorectal cancer may be underestimated. If colorectal cancer treatment is (or becomes) more expensive than modelled in this report, the cost-effectiveness of screening for Lynch syndrome will be improved.

8.1 Recommendations for research

8.1.1 Screening for Lynch syndrome in endometrial cancer patients

Given the excellent survival of endometrial cancer (compared to colorectal and ovarian cancer), there is an argument that endometrial cancer patients (either early-onset or all patients) should be screened for Lynch syndrome, since they will stand a good chance of benefitting from colorectal surveillance and other risk-reducing interventions. It is recommended that the diagnostic test accuracy of tumour-based tests should be established in endometrial tumours and the cost-effectiveness of screening should be investigated through modelling of a similar nature to that employed in this report.

8.1.2 Screening for other CRC predisposition genes in colorectal cancer patients

There are other CRC predisposition genes beyond those responsible for Lynch syndrome. It may be economical to test colorectal cancer patients for all such genes using next-generation sequencing technology, but not if tumour-based tests are used which enrich the population specifically for Lynch syndrome testing. An assessment of the yield of clinically actionable diagnoses based on large cancer risk gene panels as compared to targeted testing for Lynch syndrome-causing mutations could be conducted, along with an economic evaluation of whether such screening would be cost-effective compared to screening for Lynch syndrome (if this is recommended).

8.1.3 Costs of diagnostic tests

The cost estimates for diagnostic tests have generally been reported by genetics laboratories, rather than being formally costed. It may be worthwhile to perform a full costing exercise for these tests to determine accurate costs from an NHS and PSS perspective. The potential for cost savings through the use of next-generation sequencing technology should also be assessed.

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APPENDICES

Appendix 1. Literature search strategies

A1.1 Test accuracy searches

MEDLINE

Database	MEDLINE
Host	Ovid
Data Parameters	1946 to January Week 3 2016
Date Searched	1 February 2016
Searcher	SB
Hits	1,274

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. Colorectal Neoplasms, Hereditary Nonpolyposis/
14. or/4-13
15. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
16. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
17. 15 and 16
18. Amsterdam criteria.tw.
19. 3 or 14 or 17 or 18
20. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.
21. (Bethesda adj3 (marker* or panel*)).tw.

22. (immunohistochemistry or (IHC adj3 test*)).tw.

23. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.

24. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.

25. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.

26. exp Immunohistochemistry/

27. or/20-26

28. 19 and 27

29. exp animals/ not humans.sh.

30. 28 not 29

31. limit 30 to (english language and yr="2006 -Current")

MEDLINE In-Process & Other Non-indexed Citations

Database	MEDLINE In-Process & Other Non-Indexed Citations
Host	Ovid
Data Parameters	January 29, 2016
Date Searched	1 February 2016
Searcher	SB
Hits	134

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. or/4-12
14. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
15. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
16. 14 and 15
17. Amsterdam criteria.tw.
18. 3 or 13 or 16 or 17
19. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.
20. (Bethesda adj3 (marker* or panel*)).tw.
21. (immunohistochemistry or (IHC adj3 test*)).tw.
22. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.
23. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.

24. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.

25. or/19-24

26. 18 and 25

27. limit 26 to (english language and yr="2006 -Current")

Embase

Database	Embase
Host	Ovid
Data Parameters	1974 to 2016 January 29
Date Searched	1 February 2016
Searcher	SB
Hits	2,928

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. hereditary nonpolyposis colorectal cancer/
14. or/4-13
15. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
16. protein MLH1/
17. protein MSH2/
18. protein MSH6/
19. mismatch repair protein PMS2/
20. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
21. or/15-19
22. 20 and 21
23. Amsterdam criteria.tw.

24. 3 or 14 or 22 or 23

25. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.

26. (Bethesda adj3 (marker* or panel*)).tw.

27. (immunohistochemistry or (IHC adj3 test*)).tw.

28. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.

29. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.

30. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.

31. microsatellite instability/

32. exp Immunohistochemistry/

33. or/25-32

34. 24 and 33

35. limit 34 to (english language and yr="2006 -Current")

Web of Science

Database	Web of Science (SCI and CPCI-S)
Host	Thomson Reuters
Data Parameters	N/A
Date Searched	1 February 2016
Searcher	SB
Hits	2,335

Strategy:

1. TS=(lynch* near/2 syndrome)
2. TS=((lynch* near/2 famil*) and (cancer* or neoplasm*))
3. #2 OR #1
4. TS=("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer")
5. TS=(HNPCC)
6. TS=(((hereditary or inherited) near/2 (colon* or colorectal*)) and (cancer or neoplasm*))
7. TS=((hereditary near/2 nonpolyposis) and (colon* or colorectal*))
8. TS=((hereditary near/2 non-polyposis) and (colon* or colorectal*))
9. TS=((hereditary near/2 (cancer or neoplasm*)) and (colon* or colorectal*))
10. TS=((Familial near/2 Nonpolyposis) and (colon* or colorectal*))
11. TS=((Familial near/2 Non-polyposis) and (colon* or colorectal*))
12. TS=(familial near/2 (colon* or colorectal*))
13. #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4
14. TS=(EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2)
15. TS=(colon* or colorectal* or lynch* or HNPCC or hereditary)
16. #15 AND #14
17. TS=(Amsterdam criteria)
18. #17 OR #16 OR #13 OR #3
19. TS=((microsatellite near/2 instabilit*) or (msi near/2 test*))
20. TS=(Bethesda near/2 (marker* or panel*))
21. TS=(immunohistochemistry or (IHC near/2 test*))
22. TS=((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/2 antibod*)
23. TS=((BRAFV600E or "BRAF V600E") near/2 mutation*)

24. TS=(MLH1 near/2 (methylation or hypermethylation or "hyper methylation"))
25. #24 OR #23 OR #22 OR #21 OR #20 OR #19
26. (#25 AND #18) AND LANGUAGE: (English)

    Timespan=2006-2016

Cochrane Library

Database	Cochrane Library (Cochrane Database of Systematic Reviews [CDSR]; CENTRAL; HTA)
Host	Cochrane Collaboration
Data Parameters	CDSR and CENTRAL: Issue 1 of 12, January 2016 HTA: Issue 1 of 4, January 2016
Date Searched	1 February 2016
Searcher	SB
Hits	CDSR=0 CENTRAL=9 HTA=0

Strategy:

1. (lynch* near/3 syndrome):ti or (lynch* near/3 syndrome):ab
2. ((lynch* near/3 famil*) and (cancer* or neoplasm*)):ti or ((lynch* near/3 famil*) and (cancer* or neoplasm*)):ab
3. #1 or #2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer"):ti or ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer"):ab
5. HNPCC:ti or HNPCC:ab
6. (((hereditary or inherited) near/3 (colon* or colorectal*)) and (cancer or neoplasm*)):ti or (((hereditary or inherited) near/3 (colon* or colorectal*)) and (cancer or neoplasm*)):ab
7. ((hereditary near/3 nonpolyposis) and (colon* or colorectal*)):ti or ((hereditary near/3 nonpolyposis) and (colon* or colorectal*)):ab
8. ((hereditary near/3 non-polyposis) and (colon* or colorectal*)):ti or ((hereditary near/3 non-polyposis) and (colon* or colorectal*)):ab
9. ((hereditary near/3 (cancer or neoplasm*)) and (colon* or colorectal*)):ti or ((hereditary near/3 (cancer or neoplasm*)) and (colon* or colorectal*)):ab
10. ((Familial near/3 Nonpolyposis) and (colon* or colorectal*)):ti or ((Familial near/3 Nonpolyposis) and (colon* or colorectal*)):ab
11. ((Familial near/3 Non-polyposis) and (colon* or colorectal*)):ti or ((Familial near/3 Non-polyposis) and (colon* or colorectal*)):ab
12. (familial near/3 (colon* or colorectal*)):ti or (familial near/3 (colon* or colorectal*)):ab
13. MeSH descriptor: [Colorectal Neoplasms, Hereditary Nonpolyposis] this term only
14. {or #4-#13}
15. ((EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) and (colon* or colorectal* or lynch* or HNPCC or hereditary)):ti or ((EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) and (colon* or colorectal* or lynch* or HNPCC or hereditary)):ab

16. Amsterdam criteria:ti or Amsterdam criteria:ab

17. #15 or #16

18. #3 or #14 or #17

19. ((microsatellite near/3 instabilit*) or (msi near/3 test*)):ti or ((microsatellite near/3 instabilit*) or (msi near/3 test*)):ab

20. (Bethesda near/3 (marker* or panel*)):ti or (Bethesda near/3 (marker* or panel*)):ab

21. (immunohistochemistry or (IHC near/3 test*)):ti or (immunohistochemistry or (IHC near/3 test*)):ab

22. (MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/3 antibod*:ti or (MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/3 antibod*:ab

23. ((BRAFV600E or "BRAF V600E") near/3 mutation*):ti or ((BRAFV600E or "BRAF V600E") near/3 mutation*):ab

24. (MLH1 near/3 (methylation or hypermethylation or "hyper methylation")):ti or (MLH1 near/3 (methylation or hypermethylation or "hyper methylation")):ab

25. MeSH descriptor: [Immunohistochemistry] explode all trees

26. {or #19-#25}

27. #18 and #26 Publication Year from 2006, in Cochrane Reviews (Reviews and Protocols), Trials and Technology Assessments

Health Management Information Consortium

Database	Health Management Information Consortium
Host	Ovid
Data Parameters	1979 to November 2015
Date Searched	1 February 2016
Searcher	SB
Hits	2

Strategy: See strategy for MEDLINE In-Process & Other Non-Indexed Citations

Number of hits per database and in total

Database	Hits
MEDLINE	1,274
MEDLINE-in-Process	134
Embase	2,928
Web of Science (SCI and SCCI)	2,335
CDSR	0
CENTRAL	9
HTA	0
HMIC	2
Total records	**6,682**
Duplicates	**2,762**
Total unique records	**3,920**

A1.2 Cost effectiveness searches

MEDLINE

Database	MEDLINE
Host	Ovid
Data Parameters	1946 to January Week 3 2016
Date Searched	1 February 2016
Searcher	SB
Hits	85

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. Colorectal Neoplasms, Hereditary Nonpolyposis/
14. or/4-13
15. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
16. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
17. 15 and 16
18. Amsterdam criteria.tw.
19. 3 or 14 or 17 or 18
20. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.
21. (Bethesda adj3 (marker* or panel*)).tw.
22. (immunohistochemistry or (IHC adj3 test*)).tw.

23. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.

24. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.

25. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.

26. exp Immunohistochemistry/

27. or/20-26

28. exp Economics/

29. ec.fs.

30. economics, medical/

31. economics, nursing/

32. economics, pharmaceutical/

33. exp "economics, hospital"/

34. (economic* or price or prices or pricing or priced or discount or discounts or discounted or discounting or ration* or expenditure or expenditures or budget* or afford* or pharmacoeconomic or pharmaco-economic*).tw.

35. (cba or cea or cua).ti,ab.

36. exp "fees and charges"/

37. (fee or fees or charge* or preference*).tw.

38. (fiscal or funding or financial or finance).tw.

39. exp "costs and cost analysis"/

40. exp Health Care Costs/

41. cost*.tw.

42. exp decision support techniques/

43. exp models, economic/

44. exp Statistical Model/

45. markov*.tw.

46. markov chains/

47. monte carlo.tw.

48. monte carlo method/

49. (decision adj2 (tree* or analy* or model*)).tw.

50. (survival adj3 analys*).tw.

51. "deductibles and coinsurance"/

52. exp Health expenditures/
53. uncertain*.tw.
54. uncertainty/
55. (quality adj3 life).tw.
56. quality of life/
57. value of life/
58. Quality-adjusted life years/
59. (qol* or qoly or qolys or hrqol* or qaly or qalys or qale or qales).tw.
60. (sensitivity analys* or "willingness to pay" or quality-adjusted life year* or quality adjusted life year* or quality-adjusted life expectanc* or quality adjusted life expectanc*).tw.
61. utilit*.tw.
62. valu*.tw.
63. exp hospitalization/
64. or/28-63
65. 19 and 27 and 64
66. Animals/ not humans.sh.
67. 65 not 66
68. limit 67 to (english language and yr="2013 -Current")

MEDLINE In-Process & Other Non-Indexed Citations

Database	MEDLINE In-Process & Other Non-Indexed Citations
Host	Ovid
Data Parameters	January 29, 2016
Date Searched	1 February 2016
Searcher	SB
Hits	30

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. or/4-12
14. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
15. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
16. 14 and 15
17. Amsterdam criteria.tw.
18. 3 or 13 or 16 or 17
19. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.
20. (Bethesda adj3 (marker* or panel*)).tw.
21. (immunohistochemistry or (IHC adj3 test*)).tw.
22. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.
23. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.

24. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.

25. or/19-24

26. (economic* or price or prices or pricing or priced or discount or discounts or discounted or discounting or ration* or expenditure or expenditures or budget* or afford* or pharmacoeconomic or pharmaco-economic*).tw.

27. (cba or cea or cua).ti,ab.

28. (fee or fees or charge* or preference*).tw.

29. (fiscal or funding or financial or finance).tw.

30. cost*.tw.

31. markov*.tw.

32. monte carlo.tw.

33. (decision adj2 (tree* or analy* or model*)).tw.

34. (survival adj3 analys*).tw.

35. uncertain*.tw.

36. (quality adj3 life).tw.

37. (qol* or qoly or qolys or hrqol* or qaly or qalys or qale or qales).tw.

38. (sensitivity analys* or "willingness to pay" or quality-adjusted life year* or quality adjusted life year* or quality-adjusted life expectanc* or quality adjusted life expectanc*).tw.

39. utilit*.tw.

40. valu*.tw.

41. or/25-40

42. 18 and 25 and 41

43. limit 42 to yr="2013 -Current"

Embase

Database	Embase
Host	Ovid
Data Parameters	1974 to 2016 January 29
Date Searched	1 February 2016
Searcher	SB
Hits	256

Strategy:

1. (lynch* adj3 syndrome).tw.
2. ((lynch* adj3 famil*) and (cancer* or neoplasm*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon* or colorectal*)) and (cancer or neoplasm*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon* or colorectal*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon* or colorectal*)).tw.
9. ((hereditary adj3 (cancer or neoplasm*)) and (colon* or colorectal*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon* or colorectal*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon* or colorectal*)).tw.
12. (familial adj3 (colon* or colorectal*)).tw.
13. hereditary nonpolyposis colorectal cancer/
14. or/4-13
15. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
16. protein MLH1/
17. protein MSH2/
18. protein MSH6/
19. mismatch repair protein PMS2/
20. (colon* or colorectal* or lynch* or HNPCC or hereditary).tw.
21. or/15-19
22. 20 and 21
23. Amsterdam criteria.tw.

24. 3 or 14 or 22 or 23
25. ((microsatellite adj3 instabilit*) or (msi adj3 test*)).tw.
26. (Bethesda adj3 (marker* or panel*)).tw.
27. (immunohistochemistry or (IHC adj3 test*)).tw.
28. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod*).tw.
29. ((BRAFV600E or "BRAF V600E") adj3 mutation*).tw.
30. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.
31. microsatellite instability/
32. exp Immunohistochemistry/
33. or/25-32
34. exp Economics/
35. pe.fs.
36. economics, medical/
37. economics, nursing/
38. economics, pharmaceutical/
39. exp "economics, hospital"/
40. (economic* or price or prices or pricing or priced or discount or discounts or discounted or discounting or ration* or expenditure or expenditures or budget* or afford* or pharmacoeconomic or pharmaco-economic*).tw.
41. (cba or cea or cua).ti,ab.
42. exp "fees and charges"/
43. (fee or fees or charge* or preference*).tw.
44. (fiscal or funding or financial or finance).tw.
45. exp "costs and cost analysis"/
46. exp Health Care Costs/
47. cost*.tw.
48. exp decision support techniques/
49. exp models, economic/
50. exp Statistical Model/
51. markov*.tw.
52. markov chains/

53. monte carlo.tw.
54. monte carlo method/
55. (decision adj2 (tree* or analy* or model*)).tw.
56. (survival adj3 analys*).tw.
57. "deductibles and coinsurance"/
58. exp Health expenditures/
59. uncertain*.tw.
60. uncertainty/
61. (quality adj3 life).tw.
62. quality of life/
63. value of life/
64. Quality-adjusted life years/
65. (qol* or qoly or qolys or hrqol* or qaly or qalys or qale or qales).tw.
66. (sensitivity analys* or "willingness to pay" or quality-adjusted life year* or quality adjusted life year* or quality-adjusted life expectanc* or quality adjusted life expectanc*).tw.
67. utilit*.tw.
68. valu*.tw.
69. exp hospitalization/
70. or/34-69
71. 24 and 33 and 70
72. limit 71 to (english language and yr="2013 -Current")

Web of Science

Database	Web of Science (SCI and CPCI-S)
Host	Thomson Reuters
Data Parameters	N/A
Date Searched	1 February 2016
Searcher	SB
Hits	183

Strategy:

1. TS=(lynch* near/2 syndrome)
2. TS=((lynch* near/2 famil*) and (cancer* or neoplasm*))
3. #2 OR #1
4. TS=("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer")
5. TS=(HNPCC)
6. TS=(((hereditary or inherited) near/2 (colon* or colorectal*)) and (cancer or neoplasm*))
7. TS=((hereditary near/2 nonpolyposis) and (colon* or colorectal*))
8. TS=((hereditary near/2 non-polyposis) and (colon* or colorectal*))
9. TS=((hereditary near/2 (cancer or neoplasm*)) and (colon* or colorectal*))
10. TS=((Familial near/2 Nonpolyposis) and (colon* or colorectal*))
11. TS=(familial near/2 (colon* or colorectal*))
12. TS=((Familial near/2 Non-polyposis) and (colon* or colorectal*))
13. #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4
14. TS=(EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2)
15. TS=(colon* or colorectal* or lynch* or HNPCC or hereditary)
16. #15 AND #14
17. TS=(Amsterdam criteria)
18. #17 OR #16 OR #13 OR #3
19. TS=((microsatellite near/2 instabilit*) or (msi near/2 test*))
20. TS=(Bethesda near/2 (marker* or panel*))
21. TS=(immunohistochemistry or (IHC near/2 test*))
22. TS=((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/2 antibod*)
23. TS=((BRAFV600E or "BRAF V600E") near/2 mutation*)

24. TS=(MLH1 near/2 (methylation or hypermethylation or "hyper methylation"))

25. #24 OR #23 OR #22 OR #21 OR #20 OR #19

26. TS=(economic* or price or prices or pricing or priced or discount or discounts or discounted or discounting or ration* or expenditure or expenditures or budget* or afford* or pharmacoeconomic or pharmaco-economic*)

27. TS=(cba or cea or cua)

28. TS=(fee or fees or charge* or preference*)

29. TS=(fiscal or funding or financial or finance)

30. TS=(cost*)

31. TS=(markov*)

32. TS=(monte carlo)

33. TS=(decision near/1 (tree* or analy* or model*))

34. TS=(survival near/2 analys*)

35. TS=(uncertain*)

36. TS=(quality near/2 life)

37. TS=(qol* or qoly or qolys or hrqol* or qaly or qalys or qale or qales)

38. TS=(sensitivity analys* or "willingness to pay" or quality-adjusted life year* or quality adjusted life year* or quality-adjusted life expectanc* or quality adjusted life expectanc*)

39. TS=(utilit*)

40. TS=(valu*)

41. #40 OR #39 OR #38 OR #37 OR #36 OR #35 OR #34 OR #33 OR #32 OR #31 OR #30 OR #29 OR #28 OR #27 OR #26

42. #18 AND #25 AND #41

Timespan=2013-2016

NHS EED

Database	NHS EED
Host	Cochrane Library
Data Parameters	Issue 2 of 12, February 2016
Date Searched	1 February 2016
Searcher	SB
Hits	0

Strategy:

1. (lynch* near/3 syndrome):ti or (lynch* near/3 syndrome):ab in Economic Evaluations
2. ((lynch* near/3 famil*) and (cancer* or neoplasm*)):ti or ((lynch* near/3 famil*) and (cancer* or neoplasm*)):ab in Economic Evaluations
3. #1 or #2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer"):ti or ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer"):ab in Economic Evaluations
5. HNPCC:ti or HNPCC:ab in Economic Evaluations
6. (((hereditary or inherited) near/3 (colon* or colorectal*)) and (cancer or neoplasm*)):ti or (((hereditary or inherited) near/3 (colon* or colorectal*)) and (cancer or neoplasm*)):ab in Economic Evaluations
7. ((hereditary near/3 nonpolyposis) and (colon* or colorectal*)):ti or ((hereditary near/3 nonpolyposis) and (colon* or colorectal*)):ab in Economic Evaluations
8. ((hereditary near/3 non-polyposis) and (colon* or colorectal*)):ti or ((hereditary near/3 non-polyposis) and (colon* or colorectal*)):ab in Economic Evaluations
9. ((hereditary near/3 (cancer or neoplasm*)) and (colon* or colorectal*)):ti or ((hereditary near/3 (cancer or neoplasm*)) and (colon* or colorectal*)):ab in Economic Evaluations
10. ((Familial near/3 Nonpolyposis) and (colon* or colorectal*)):ti or ((Familial near/3 Nonpolyposis) and (colon* or colorectal*)):ab in Economic Evaluations
11. ((Familial near/3 Non-polyposis) and (colon* or colorectal*)):ti or ((Familial near/3 Non-polyposis) and (colon* or colorectal*)):ab in Economic Evaluations
12. (familial near/3 (colon* or colorectal*)):ti or (familial near/3 (colon* or colorectal*)):ab in Economic Evaluations
13. MeSH descriptor: [Colorectal Neoplasms, Hereditary Nonpolyposis] this term only
14. {or #4-#13} in Economic Evaluations
15. ((EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) and (colon* or colorectal* or lynch* or HNPCC or hereditary)):ti or ((EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) and (colon* or colorectal* or lynch* or HNPCC or hereditary)):ab in Economic Evaluations
16. Amsterdam criteria:ti or Amsterdam criteria:ab in Economic Evaluations

17. #15 or #16

18. #3 or #14 or #17

19. ((microsatellite near/3 instabilit*) or (msi near/3 test*)):ti or ((microsatellite near/3 instabilit*) or (msi near/3 test*)):ab in Economic Evaluations

20. (Bethesda near/3 (marker* or panel*)):ti or (Bethesda near/3 (marker* or panel*)):ab in Economic Evaluations

21. (immunohistochemistry or (IHC near/3 test*)):ti or (immunohistochemistry or (IHC near/3 test*)):ab in Economic Evaluations

22. (MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/3 antibod*:ti or (MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) near/3 antibod*:ab in Economic Evaluations

23. ((BRAFV600E or "BRAF V600E") near/3 mutation*):ti or ((BRAFV600E or "BRAF V600E") near/3 mutation*):ab in Economic Evaluations

24. (MLH1 near/3 (methylation or hypermethylation or "hyper methylation")):ti or (MLH1 near/3 (methylation or hypermethylation or "hyper methylation")):ab in Economic Evaluations

25. MeSH descriptor: [Immunohistochemistry] explode all trees

26. {or #19-#25}

27. #18 and #26 Publication Year from 2013 to 2016, in Economic Evaluations

EconLit

Database	EconLit
Host	EBSCO
Data Parameters	N/A
Date Searched	1 February 2016
Searcher	SB
Hits	2

Strategy:

1. TI lynch* N2 syndrome OR AB lynch* N2 syndrome
2. TI ((lynch* N2 famil*) AND (cancer* or neoplasm*)) OR AB ((lynch* N2 famil*) AND (cancer* or neoplasm*))
3. S1 OR S2
4. TI ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer") OR AB ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer")
5. TI HNPCC OR AB HNPCC
6. TI (((hereditary or inherited) N2 (colon* or colorectal*)) and (cancer or neoplasm*)) OR AB (((hereditary or inherited) N2 (colon* or colorectal*)) and (cancer or neoplasm*))
7. TI ((hereditary N2 nonpolyposis) and (colon* or colorectal*)) OR AB ((hereditary N2 nonpolyposis) and (colon* or colorectal*))
8. TI ((hereditary N2 non-polyposis) and (colon* or colorectal*)) OR AB ((hereditary N2 non-polyposis) and (colon* or colorectal*))
9. TI ((hereditary N2 (cancer or neoplasm*)) and (colon* or colorectal*)) OR AB ((hereditary N2 (cancer or neoplasm*)) and (colon* or colorectal*))
10. TI ((Familial N2 Nonpolyposis) and (colon* or colorectal*)) OR AB ((Familial N2 Nonpolyposis) and (colon* or colorectal*))
11. TI (((Familial N2 Non-polyposis) and (colon* or colorectal*))) OR AB ((Familial N2 Non-polyposis) and (colon* or colorectal*))
12. TI ((familial N2 (colon* or colorectal*))) OR AB ((familial N2 (colon* or colorectal*)))
13. S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12
14. TI (EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) OR AB (EPCAM* or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2)
15. TI (colon* or colorectal* or lynch* or HNPCC or hereditary) AND AB (colon* or colorectal* or lynch* or HNPCC or hereditary)
16. S14 AND S15
17. TI Amsterdam criteria OR AB Amsterdam criteria
18. S3 OR S13 OR S16 OR S17

19. TI ((microsatellite N2 instabilit*) or (msi N2 test*)) OR AB ((microsatellite N2 instabilit*) or (msi N2 test*))

20. TI ((Bethesda N2 (marker* or panel*))) OR AB ((Bethesda N2 (marker* or panel*)))

21. TI (immunohistochemistry or (IHC N2 test*)) OR AB (immunohistochemistry or (IHC N2 test*))

22. TI ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) N2 antibod*) OR AB ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) N2 antibod*)

23. TI ((BRAFV600E or "BRAF V600E") N2 mutation*) OR AB ((BRAFV600E or "BRAF V600E") N2 mutation*)

24. TI ((MLH1 N2 (methylation or hypermethylation or "hyper methylation"))) OR AB ((MLH1 N2 (methylation or hypermethylation or "hyper methylation")))

25. S19 OR S20 OR S21 OR S22 OR S23 OR S24

26. S18 AND S25

Number of hits per database and in total

Database	Hits
MEDLINE	85
MEDLINE-in-Process	30
Embase	256
Web of Science (SCI and SCCI)	183
NHS EED	0
EconLit	2
Total records	**556**
Duplicates	**204**
Total unique records	**352**

A1.3 Utilities searches

A1.3.1 Hysterectomy and salpingo-oophorectomy

MEDLINE and MEDLINE In-Process

Database	MEDLINE and MEDLINE-in-Process
Host	Ovid
Data Parameters	1946 to Present
Date Searched	25 February 2016
Searcher	SB
Hits	13

Strategy:

1. hysterectom*.tw.
2. ("salpingo oophorectom*" or "salpingo ovariectom*" or salpingooophorectom*).tw.
3. (salpingectom* adj7 (oophorectom* or ovariectom*)).tw.
4. 1 and (2 or 3)
5. exp Hysterectomy/
6. Salpingectomy/
7. Ovariectomy/
8. and/5-7
9. 4 or 8
10. (HRQOL or HRQL or QOL or QALY*).tw.
11. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
12. 10 or 11
13. 9 and 12
14. exp animals/ not humans.sh.
15. 13 not 14
16. limit 15 to english language

Embase

Database	Embase
Host	Ovid
Data Parameters	1974 to 2016 February 24
Date Searched	25 February 2016
Searcher	SB
Hits	27

Strategy:

1. hysterectom*.tw.
2. ("salpingo oophorectom*" or "salpingo ovariectom*" or salpingooophorectom*).tw.
3. (salpingectom* adj7 (oophorectom* or ovariectom*)).tw.
4. 1 and (2 or 3)
5. exp hysterectomy/
6. salpingooophorectomy/
7. and/5-6
8. 4 or 7
9. (HRQOL or HRQL or QOL or QALY*).tw.
10. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
11. 9 or 10
12. 8 and 11
13. Limit 12 to english language

A1.3.2 Colorectal cancer

MEDLINE and MEDLINE In-Process

Database	MEDLINE and MEDLINE-in-Process
Host	Ovid
Data Parameters	1946 to Present
Date Searched	25 February 2016
Searcher	SB
Hits	757

Strategy:

1. ((colorectal or colon or colorectum or colonic or rectal or rectum or bowel or intenstin*) adj3 (cancer* or carcinom* or neoplasm* or malignan* or tumo?r*)).tw.
2. (CRC or mCRC).tw.
3. exp Colorectal Neoplasms/
4. or/1-3
5. (HRQOL or HRQL or QOL or QALY*).tw.
6. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
7. ("QLQ-C30" or "FACT-G").tw.
8. or/5-7
9. 4 and 8
10. exp animals/ not humans.sh.
11. 9 not 10
12. limit 11 to (english language and yr="2005 -Current")

Embase

Database	Embase
Host	Ovid
Data Parameters	1974 to 2016 February 24
Date Searched	25 February 2016
Searcher	SB
Hits	1,833

Strategy:

1. ((colorectal or colon or colorectum or colonic or rectal or rectum or bowel or intenstin*) adj3 (cancer* or carcinom* or neoplasm* or malignan* or tumo?r*)).tw.
2. (CRC or mCRC).tw.
3. exp colon tumor/
4. exp rectum tumor/
5. or/1-4
6. (HRQOL or HRQL or QOL or QALY*).tw.
7. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
8. ("QLQ-C30" or "FACT-G").tw.
9. or/6-8
10. 5 and 9
11. limit 10 to (english language and yr="2005 -Current")

A1.3.3 Endometrial cancer

MEDLINE and MEDLINE In-Process

Database	MEDLINE and MEDLINE In-Process
Host	Ovid
Data Parameters	1946 to Present
Date Searched	25 February 2016
Searcher	SB
Hits	137

Strategy:

1. ((endometrial or endometrium or uterine or uterus) adj3 (cancer* or carcinom* or neoplasm* or malignan* or tumo?r*)).tw.
2. exp Endometrial Neoplasms/
3. 1 or 2
4. (HRQOL or HRQL or QOL or QALY*).tw.
5. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
6. ("QLQ-C30" or "FACT-G").tw.
7. or/4-6
8. 3 and 7
9. exp animals/ not humans.sh.
10. 8 not 9
11. limit 10 to english language

Embase

Database	Embase
Host	Ovid
Data Parameters	1974 to 2016 February 24
Date Searched	25 February 2016
Searcher	SB
Hits	330

Strategy:

1. ((endometrial or endometrium or uterine or uterus) adj3 (cancer* or carcinom* or neoplasm* or malignan* or tumo?r*)).tw.
2. exp endometrium tumor/
3. 1 or 2
4. (HRQOL or HRQL or QOL or QALY*).tw.
5. (EQ-5D or EQ-5D-3L or EQ-5D-5L or EQ-VAS or SF-6D or SF-12 or SF-36 or HUI2 or HUI3 or 15D or PROMIS).tw.
6. ("QLQ-C30" or "FACT-G").tw.
7. or/4-6
8. 3 and 7
9. limit 8 to english language

Number of hits per database and in total

Database	Hits
MEDLINE and MEDLINE-in-Process (hysterectomy)	13
Embase (hysterectomy)	27
MEDLINE and MEDLINE-in-Process (colorectal cancer)	757
Embase (colorectal cancer)	1,833
MEDLINE and MEDLINE-in-Process (endometrial cancer)	137
Embase (endometrial cancer)	330
Total hits	**3,097**
Duplicate hits	**906**
Unique hits	**2,191**

A1.4 Forward citation chasing on included test accuracy studies

Database	Scopus
Host	Elsevier
Data Parameters	N/A
Date Searcher	14 April 2016
Searcher	SB

Number of hits per study and in total

Study	Hits
Barnetson 2006	258
Hendriks 2003	132
Limburg 2011	24
Niessen 2006	64
Okkels 2012	5
Overbeek 2007	23
Poynter 2008	97
Shia 2005	85
Southey 2005	137
Total hits	**825**
Duplicate hits	**138**
Duplicate hits in search results for test accuracy studies	**118**
Unique hits	**569**

Appendix 2. Data extraction form

Design	Participants	Tests	Outcomes
Main paper Authors (date): **Related references:** **Basic design:** ☐ Single gate ☐ Two gate ☐ Other If other *please describe*: **Location:** **No. of centres:** **Funding:**	**No. recruited:** **Type of participants:** ☐ All CRC ☐ Age limited If age limited *please describe*: ☐ 'High risk' If 'high risk' *please describe*, including selection due to prior testing: **Selection:** ☐ Consecutive ☐ Random ☐ Unclear ☐ Other If other *please describe*: **Participant inclusion criteria:** **Participant exclusion criteria:**	**Index tests included:** ☐ MSI - With BRAF V600E test ☐ - With *MLH1* methylation test ☐ *Please list* panel of markers: ☐ IHC - With BRAF V600E test ☐ - With *MLH1* methylation test ☐ *Please list* proteins: **Reference standard description and notes:** **Reference standard genes:** ☐ *MLH1* ☐ *MSH2* ☐ *MSH6* If other genes tested, *please list*: **Reference standard testing for large genomic mutations:** ☐ MLPA ☐ Other If other, *please describe*: **Time intervals between tests:**	**Accuracy outcomes reported :** ☐ Sensitivity ☐ Specificity ☐ (LR+) ☐ (LR−) ☐ PPV ☐ NPV ☐ Diagnostic yield/test positivity rate/apparent prevalence ☐ Test failure rate **Data** **Sample attrition / dropout:** **No. receiving index test (and reasons):** **No. receiving reference standard (and reasons):** **Data excluded (and reasons):**
Notes			

Participant characteristics

	Index test 1	Index test 2	Index test 3	Reference standard
No. of patients				
Median/mean age, yrs (range)				
No. <50years				
No. meeting AMS II				
No. meeting Bethesda				
Gender				
- Men				
- Women				
Ethnicity				
-				
-				

Cancer location				
- Rectum				
- Left colon				
- Right colon				
- Transverse colon				

Results (copy more tables as needed)

MSI v reference standard: ☐ with BRAF V600E test ☐ with MLH1 methylation test

MSI	Reference standard		
	+ve	-ve	Total
+ve			
-ve			
Total			

IHC v reference standard: ☐ with BRAF V600E test ☐ with MLH1 methylation test

IHC	Reference standard		
	+ve	-ve	Total
+ve			
-ve			
Total			

Other key results:

Quality appraisal – QUADAS-2 (Phase 3)

DOMAIN 1: PATIENT SELECTION

Risk of bias:

- Was a consecutive or random sample of patients enrolled? (Y/N/U)
- Was a case-control study design avoided? (Y/N/U)
- Did the study avoid inappropriate exclusions? (Y/N/U)

Could the selection of patients have introduced bias? (H/L/U)

Concerns regarding applicability:

Is there concern that the included patients do not match the review question? (H/L/U)

DOMAIN 2: INDEX TESTS (complete for each index test)

Risk of bias:

- Were the index test results interpreted without knowledge of the results of the reference standard? (Y/N/U)
- If a threshold was used, was it pre-specified? (Y/N/U)

Could the conduct or interpretation of the index test have introduced bias? (H/L/U)

Concerns regarding applicability:

Is there concern that the index test, its conduct, or interpretation differ from the review question? (H/L/U)

DOMAIN 3: REFERENCE STANDARD

Risk of bias:

- Is the reference standard likely to correctly classify the target condition? (Y/N/U)

- Were the reference standard results interpreted without knowledge of the results of the index test?	(Y/N/U)
Could the reference standard, its conduct, or its interpretation have introduced bias?	(H/L/U)
Concerns regarding applicability:	
Is there concern that the target condition as defined by the reference standard does not match the review question?	(H/L/U)
DOMAIN 4 : FLOW AND TIMING	
Risk of bias:	
- Was there an appropriate interval between index test(s) and reference standard?	(Y/N/U)
- Did all patients receive a reference standard?	(Y/N/U)
- Did all patients receive the same reference standard?	(Y/N/U)
- Were all patients included in the analysis?	(Y/N/U)
Could the patient flow have introduced bias?	(H/L/U)
Quality appraisal – Additional notes	
Add any notes to necessary to explain ratings above, or anything relevant to risk of bias not covered above:	

Appendix 3. Quality assessment

Quality appraisal was performed using Phase 3 of the QUADAS-2 (www.quadas.org):

Phase 3: Risk of bias and applicability judgments

QUADAS-2 is structured so that 4 key domains are each rated in terms of the risk of bias and the concern regarding applicability to the research question (as defined above). Each key domain has a set of signalling questions to help reach the judgments regarding bias and applicability.

DOMAIN 1: PATIENT SELECTION

A. Risk of Bias

Describe methods of patient selection:

- ❖ Was a consecutive or random sample of patients enrolled? Yes/No/Unclear
- ❖ Was a case-control design avoided? Yes/No/Unclear
- ❖ Did the study avoid inappropriate exclusions? Yes/No/Unclear

Could the selection of patients have introduced bias? RISK: LOW/HIGH/UNCLEAR

B. Concerns regarding applicability

Describe included patients (prior testing, presentation, intended use of index test and setting):

Is there concern that the included patients do not match the review question? CONCERN: LOW/HIGH/UNCLEAR

DOMAIN 2: INDEX TEST(S)

If more than one index test was used, please complete for each test.

A. Risk of Bias

Describe the index test and how it was conducted and interpreted:

- ❖ Were the index test results interpreted without knowledge of the results of the reference standard? Yes/No/Unclear
- ❖ If a threshold was used, was it pre-specified? Yes/No/Unclear

Could the conduct or interpretation of the index test have introduced bias? RISK: LOW /HIGH/UNCLEAR

B. Concerns regarding applicability

Is there concern that the index test, its conduct, or interpretation differ from the review question? CONCERN: LOW /HIGH/UNCLEAR

DOMAIN 3: REFERENCE STANDARD

A. Risk of Bias

Describe the reference standard and how it was conducted and interpreted:

- ❖ Is the reference standard likely to correctly classify the target condition? Yes/No/Unclear
- ❖ Were the reference standard results interpreted without knowledge of the results of the index test? Yes/No/Unclear

Could the reference standard, its conduct, or its interpretation have introduced bias? **RISK: LOW /HIGH/UNCLEAR**

B. Concerns regarding applicability

Is there concern that the target condition as defined by the reference standard does not match the review question? **CONCERN: LOW /HIGH/UNCLEAR**

DOMAIN 4: FLOW AND TIMING

A. Risk of Bias

Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):

Describe the time interval and any interventions between index test(s) and reference standard:

- ❖ Was there an appropriate interval between index test(s) and reference standard? Yes/No/Unclear
- ❖ Did all patients receive a reference standard? Yes/No/Unclear
- ❖ Did patients receive the same reference standard? Yes/No/Unclear
- ❖ Were all patients included in the analysis? Yes/No/Unclear

Could the patient flow have introduced bias? **RISK: LOW /HIGH/UNCLEAR**

Appendix 4. Table of excluded studies with rationale

Studies excluded at the full text stage came from four sources: electronic searches (n=71), searching systematic reviews (n=38), forward citation chasing (n=4), and backward citation chasing (n=11). These studies are listed below. Reasons for exclusion are as follows:

- Population – the study did not recruit the population specified in the protocol
- Index test - there was no index test or the study did not use an index test as specified in the protocol
- Reference standard – there was no reference standard, or the study did not include a reference standard as specified in the protocol
- Outcomes - the study did not report outcomes as specified in the protocol
- Study design – the study design was not as specified in the protocol, including when the reference standard was not given to all participants (for high-risk studies) or was not given to a representative sample of index test negatives (for population-based studies)
- Abstract only – the study was published only as an abstract that was not linked to an included study that was published in full

Duplicate – the reference was a duplicate of a study that had already been assessed for inclusion but this was either missed or not evident at an earlier stage

A4.1 Excluded full texts identified from electronic searches

Reference	Primary criterion not met
Abbott, D. E., Cantor, S. B., Miguel, A. R. B., Chang, G. J., Lynch, P. M., Feig, B. W., et al. (2012). Detecting hereditary nonpolyposis colorectal cancer syndrome (HNPCC) in patients with colorectal cancer (CRC): Optimal strategies at lower costs. Journal of Clinical Oncology. Conference, 30.	Abstract only
Akagi, K., Kakuta, M., Takahashi, A., Arai, Y., Nishimura, Y., Yatsuoka, T., et al. (2011). Molecular screening with colorectal tumor tissue for Lynch syndrome. Familial Cancer, 10, S43.	Study design
Alemayehu, A., Tomkova, K., Zavodna, K., Ventusova, K., Krivulcik, T., Bujalkova, M., et al. (2007). The role of clinical criteria, genetic and epigenetic alterations in Lynch-syndrome diagnosis. Neoplasma, 54, 391-401.	Study design
Alenda, C., Paya, A., Perez, L., Alcaraz, E., Soto, J. L., Guillen, C., et al. (2009). Usefulness of p16 immunohistochemistry in the diagnosis of Lynch's syndrome. Laboratory Investigation, 89, 122A-123A.	Abstract only
Alvarez, K., Hurtado, C., Hevia, M. A., Wielandt, A. M., de la Fuente, M., Church, J., et al. (2010). Spectrum of MLH1 and MSH2 mutations in Chilean families with suspected Lynch syndrome. Diseases of the Colon & Rectum, 53, 450-459.	Study design
Barrow, E., Alduaij, W., Robinson, L., Shenton, A., Clancy, T., Lalloo, F., et al. (2008). Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. Clinical	Study design

Genetics, 74, 233-242.	
Barrow, E., Evans, D. G., McMahon, R., Byers, R., & Hill, J. (2009). A comparative study of quantitative immunohistochemistry and quantum dot immunohistochemistry for mutation carrier identification in Lynch Syndrome. Colorectal Disease, 11, 15.	Abstract only
Barrow, E., Evans, D. G., McMahon, R., Hill, J., & Byers, R. (2011). A comparative study of quantitative immunohistochemistry and quantum dot immunohistochemistry for mutation carrier identification in Lynch syndrome. Journal of Clinical Pathology, 64, 208-214.	Reference standard
Barrow, E., Jagger, E., Brierley, J., Wallace, A., Evans, G., Hill, J., et al. (2010). Semiquantitative assessment of immunohistochemistry for mismatch repair proteins in Lynch syndrome. Histopathology, 56, 331-344.	Reference standard
Bessa, X., Balleste, B., Andreu, M., Castells, A., Bellosillo, B., Balaguer, F., et al. (2008). A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. Clinical Gastroenterology & Hepatology, 6, 206-214.	Study design
Canard, G., Lefevre, J. H., Colas, C., Coulet, F., Svrcek, M., Lascols, O., et al. (2012). Screening for Lynch syndrome in colorectal cancer: are we doing enough? Annals of Surgical Oncology, 19, 809-816.	Study design
Canard, G., Lefevre, J., Colas, C., Coulet, F., Soubrier, F., Svreck, M., et al. (2011). Screening HNPCC syndrome in colorectal cancer: Are we doing enough? Colorectal Disease, 13, 13-14.	Study design
Canard, G., Lefevre, J., Colas, C., Coulet, F., Soubrier, F., Svreck, M., et al. (2010). Screening HNPCC syndrome in colorectal cancer: Are we doing enough? Colorectal Disease, 12, 13.	Study design
Castells, A., Paya, A., Alenda, C., Rodriguez-Moranta, F., Agrelo, R., Andreu, M., et al. (2006). Cyclooxygenase 2 expression in colorectal cancer with DNA mismatch repair deficiency. Clinical Cancer Research, 12, 1686-1692.	Study design
Chang, S. C., Lin, P. C., Yang, S. H., Wang, H. S., Liang, W. Y., & Lin, J. K. (2010). Taiwan hospital-based detection of Lynch syndrome distinguishes 2 types of microsatellite instabilities in colorectal cancers. Surgery, 147, 720-728.	Study design
Chew, M. H., Liu, Y. Q., & Tang, C. L. (2013). Systematic study on genetic and epimutational profile of a cohort of Amsterdam criteria-defined Lynch Syndrome in Singapore. Colorectal Disease, 15, 41.	Abstract only
De Leon, E. D., Robinson, L., Euhus, D., Burstein, E., & Sarode, V. R. (2014). Evaluation of lynch syndrome by immunohistochemistry and quantitative scoring by digital image analysis as a screening tool for the diagnosis of hereditary colon cancer and correlation with genetic analysis. Gastroenterology, 1), S-346.	Abstract only
Dong, H. S., Dong, K. C., Kim, Y. H., Rhee, P. L., Kim, J. J., Dae, S. K., et al. (2009). Effectiveness of each bethesda marker in defining microsatellite instability when screening for Lynch syndrome. Hepato-Gastroenterology, 56, 672-676.	Study design
Dudley, B., Brand, R. E., Thull, D., Bahary, N., Nikiforova, M. N., & Pai, R. K. (2015). Germline MLH1 Mutations Are Frequently Identified in Lynch Syndrome Patients With Colorectal and Endometrial Carcinoma Demonstrating Isolated Loss of PMS2 Immunohistochemical Expression. American Journal of Surgical Pathology, 39, 1114-1120.	Study design
Farrell, M. P., Clabby, C., Shea, R. O., Green, A. J., & Gallagher, D. J. (2015). MLH1 hypermethylation assay should be considered when evaluating mismatch repair deficient tumours. Journal of Clinical Oncology. Conference,	Abstract only

33.	
Gausachs, M., Mur, P., Corral, J., Pineda, M., Gonzalez, S., Benito, L., et al. (2012). MLH1 promoter hypermethylation in the analytical algorithm of Lynch syndrome: a cost-effectiveness study. European Journal of Human Genetics, 20, 762-768.	Reference standard
Gilmour, K., Azam, T., Onyeador, N., Lees, L., Walsh, K., Ingman, T., et al. (2012). Multidisciplinary stratification of colorectal cancer patients for clinical and therapeutic decision making. Journal of Pathology, 226, S24.	Reference standard
Giraldez, M. D., Balaguer, F., Cuatrecasas, M., Munoz, J., Alonso-Espinaco, V., Bujanda, L., et al. (2010). High frequency of MSH6 germline mutations in early-onset colorectal cancer. Gastroenterology, 1), S150.	Abstract only
Giraldez, M. D., Balaguer, F., Petit, A., Bujanda, L., Moyano, S., Gonzalo, V., et al. (2009). High frequency of MSH6 loss in early-onset colorectal cancer. Gastroenterology, 1), A452.	Reference standard
Goel, A., Rhees, J., Nagasaka, T., & Boland, C. R. (2010). Somatic hypermethylation of MSH2 is a frequent event in lynch syndrome colorectal cancers. Gastroenterology, 1), S150.	Outcomes
Gould-Suarez, M., El-Serag, H. B., Musher, B., Franco, L. M., & Chen, G. J. (2014). Cost-effectiveness and diagnostic effectiveness analyses of multiple algorithms for the diagnosis of Lynch syndrome. Digestive Diseases & Sciences, 59, 2913-2926.	Study design
Guarinos, C., Castillejo, A., Barbera, V. M., Carbonell, L., Sanchez-Heras, A. B., Castillejo, M. I., et al. (2010). Impact of TACSTD1 germline deletions as Lynch syndrome causing mutations in Spanish hereditary non-polyposis colorectal cancer-suspected patients. European Journal of Cancer, Supplement, 8 (5), 45.	Abstract only
Guarinos, C., Castillejo, A., Barbera, V. M., Perez-Carbonell, L., Sanchez-Heras, A. B., Segura, A., et al. (2010). EPCAM Germ Line Deletions as Causes of Lynch Syndrome in Spanish Patients. Journal of Molecular Diagnostics, 12, 765-770. doi: 10.2353/jmoldx.2010.100039	Study design
Haghighi, M. M., Mohebbi, S. R., Molaei, M., Ghiasi, S., Fatemi, R., & Zali, M. R. (2009). Microsatellite instability markers profile in patients with colorectal cancers caused by germ-line mutations in DNA mismatch repair genes. IUBMB Life, 61 (3), 309.	Reference standard
Haraldsdottir, S., Hampel, H., Frankel, W., Wu, C. S. Y., Pan, X. J., Bekaii-Saab, T. S., et al. (2014). Effect of genetic counseling on detection of Lynch syndrome (LS) in colorectal cancer (CRC) patients (pts). Journal of Clinical Oncology. Conference, 32.	Abstract only
Idris, A. F., Hooper, S. D., Farrell, M. P., Nolan, C., Clarke, R., Berkley, E., et al. (2014). Are immunohistochemical (IHC)/microsatellite instability (MSI) testing necessary as part of Lynch syndrome work-up in the era of multiplex genetic testing? Journal of Clinical Oncology. Conference, 32.	Outcomes
Kets, C. M., van Krieken, J., Hebeda, K. M., Wezenberg, S. J., Goossens, M., Brunner, H. G., et al. (2006). Very low prevalence of germline MSH6 mutations in hereditary non-polyposis colorectal cancer suspected patients with colorectal cancer without microsatellite instability. British Journal of Cancer, 95, 1678-1682. doi: 10.1038/sj.bjc.6603478	Study design
Kim, S., Kim, J., Yu, C., Chung, H., Chun, S., Lee, W., et al. (2011). Germline mutations in MLH1 and MSH2 in Korean hereditary non-polyposis colorectal cancer (HNPCC) patients. Journal of Molecular Diagnostics, 13 (6), 717.	Abstract only
Kostina, O., Rebane, E., Anderson, W., Kask, M., & Valkna, A. (2010). Molecular diagnostics strategy to identify hereditary non-polyposis colorectal	Abstract only

cancer patients. Clinical Cancer Research. Conference: AACR International Conference on Translational Cancer Medicine, 16.	
Kostina, O., Rebane, E., Kask, M., & Anderson, W. (2011). Molecular diagnostic strategy to identify families with hereditary non-polyposis colorectal cancer syndrome. European Journal of Cancer, 47, S186.	Abstract only
Kraus, C., Rau, T., Lux, P., Erlenbach-Wunsch, K., Stohr, R., Agaimy, A., et al. (2014). Detection of presumably low penetrance germ line mutations associated with colorectal cancer-results of a prospective comparative study of germ line mutation analysis by next generation sequencing and classical molecular pathology testing. Medizinische Genetik, 26 (1), 121-122.	Abstract only
Lastella, P., Patruno, M., Forte, G., Montanaro, A., Di Gregorio, C., Sabba, C., et al. (2011). Identification and surveillance of 19 Lynch syndrome families in southern Italy: report of six novel germline mutations and a common founder mutation. Familial Cancer, 10, 285-295.	Study design
Leenen, C. H. M., Dubbink, E. J., Van Lier, M. G. F., Hulspas, S. M., Kuipers, E. J., Van Leerdam, M. E., et al. (2011). Challenges and pitfalls in screening for Lynch syndrome by molecular tumor tissue analysis. Familial Cancer, 10, S29.	Duplicate
Leenen, C. H., Dubbink, E. J., Van Lier, M. G., Hulspas, S., Kuipers, E. J., Van Leerdam, M., et al. (2011). Challenges and pitfalls in screening for lynch syndrome by molecular tumor tissue analysis. Gastroenterology, 1), S352-S353.	Abstract only
Liu, Y. Q., Goh, X. W., Tan, S. Y., Chew, M. H., Tien, C. T. L., Koh, P. K., et al. (2012). Molecular detect ion of 5 mismatch repair gene defects in Singapore cohort of lynch syndrome families. Proceedings of Singapore Healthcare, 21, S347.	Abstract only
Mafnas, C., Martin, B., Ford, J., & Longacre, T. (2015). Lynch syndrome screening: Discordance in MMR and germline test results. Laboratory Investigation, 95, 177A.	Abstract only
Mahooti, S., Hampel, H., LaJeunesse, J., Sotamaa, K., de la Chapelle, A., & Frankel, W. L. (2006). MLH1 and PMS2 protein expression in 103 colorectal carcinomas with MLH1 promoter methylation and without MLH1 or PMS2 germline mutation. Modern Pathology, 19, 113A-113A.	Abstract only
Marginean, F., Landolfi, S., Hernandez, J., de torres, I., Garrido, M., Badia, D., et al. (2008). High feasibility of hmlh1, hmsh2 and hmsh6 protein expression and microsatellite instability analysis (pentaplex system) to screen patients with clinical criteria of Lynch Syndrome. Virchows Archiv, 452, S193-S194.	Reference standard
Mokarram, P., Rismanchi, M., Alizadeh Naeeni, M., Mirab Samiee, S., Paryan, M., Alipour, A., et al. (2014). Microsatellite instability typing in serum and tissue of patients with colorectal cancer: comparing real time PCR with hybridization probe and high-performance liquid chromatography. Molecular Biology Reports, 41, 2835-2844.	Reference standard
Molinari, F., Signoroni, S., Lampis, A., Bertan, C., Perrone, F., Sala, P., et al. (2014). BRAF mutation analysis is a valid tool to implement in Lynch syndrome diagnosis in patients classified according to the Bethesda guidelines. Tumori, 100, 315-320.	Study design
Monzon, J. G., Cremin, C., Armstrong, L., Nuk, J., Young, S., Horsman, D. E., et al. (2010). Validation of predictive models for germline mutations in DNA mismatch repair genes in colorectal cancer. International Journal of Cancer, 126, 930-939.	Study design
Niessen, R. C., Berends, M. J., Wu, Y., Sijmons, R. H., Hollema, H., Ligtenberg, M. J., et al. (2006). Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple	Study design

tumours associated with hereditary non-polyposis colorectal cancer. Gut, 55, 1781-1788.	
Nizam, M. Z., Gurjeet, K., Muhammad radzi, A. H., Harjinder, S., Venkatesh rn, R. N., & Ankathil, R. (2010). Mutation and protein expression analysis of mismatch repair gene, MLH1 in Malaysian lynch syndrome patients. Journal of Gastroenterology and Hepatology, 25, A37.	Reference standard
Nizam, Z. M., Gurjeet, K., Radzi, A. H. M., Harjinder, S., Venkatesh, R. N., & Ravindran, A. (2011). Germline mutation and epimutation analysis of human mismatch repair genes, mlh1 and MSH2 in Malaysian lynch syndrome patients. HUGO Journal, 1), 243.	Reference standard
Orellana, P., Wielandt, A. M., Alvarez, K., Hurtado, C., Pinto, E., Carvallo, P., et al. (2013). Analysis of point mutatiton and genomic rearrangements in MLH1, MSH2, MSH6 and PMS2 genes in Lynch sundrome: A chilean experience. Familial Cancer, 12 (4), 803-804.	Abstract only
Orellana, P., Wielandt, A. M., Alvarez, K., Pinto, E., Hurtado, C., Zarate, A. J., et al. (2010). Usefulness of immunohistochemistry and microsatellite instability in families with suspected Lynch syndrome. Colorectal Disease, 12, 2.	Abstract only
Pan, M., Hoodfar, E., Bergoffen, J. A., Fulton, R., Hofmeister, L., Chavez, A., et al. (2012). Improving detection of Lynch syndrome using a reflex immunohistochemistry algorithm for all patients with newly diagnosed colorectal cancer. Journal of Clinical Oncology. Conference: ASCO's Quality Care Symposium, 30.	Abstract only
Paya, A., Alenda, C., Perez-Carbonell, L., Rojas, E., Soto, J. L., Guillen, C., et al. (2009). Utility of p16 immunohistochemistry for the identification of Lynch syndrome. Clinical Cancer Research, 15, 3156-3162.	Study design
Perea, J., Rodriguez, Y., Rueda, D., Marin, J. C., Diaz-Tasende, J., Alvaro, E., et al. (2011). Early-onset colorectal cancer is an easy and effective tool to identify retrospectively Lynch syndrome. Annals of Surgical Oncology, 18, 3285-3291.	Study design
Perez-Cabornero, L., Infante Sanz, M., Velasco Sampedro, E., Lastra Aras, E., Acedo Becares, A., Miner Pino, C., et al. (2011). Frequency of rearrangements in Lynch syndrome cases associated with MSH2: characterization of a new deletion involving both EPCAM and the 5' part of MSH2. Cancer Prevention Research, 4, 1556-1562.	Study design
Perez-Carbonell, L., Alenda, C., Paya, A., Castillejo, A., Barbera, V. M., Guillen, C., et al. (2010). Methylation analysis of MLH1 improves the selection of patients for genetic testing in Lynch syndrome. Journal of Molecular Diagnostics, 12, 498-504.	Study design
Perez-Carbonell, L., Guarinos, C., Soler, M. R., Sanchez-Fortun, C., Sempere-Robles, L., Ruiz-Ponte, C., et al. (2011). Comparison between universal immunohistochemistry for mismatch repair proteins versus revised bethesda guidelines in the detection of patients with lynch syndrome. Gastroenterology, 1), S97.	Study design
Perez-Carbonell, L., Ruiz-Ponte, C., Bessa, X., Soto, J. L., Castillejo, A., Barbera, V., et al. (2010). Comparison between routine immunohistochemistry for mismatch repair proteins versus revised bethesda guidelines in the diagnosis of lynch syndrome in a non-selected population of colorectal cancer patients. Gastroenterology, 1), S297.	Study design
Perez-Carbonell, L., Ruiz-Ponte, C., Guarinos, C., Alenda, C., Paya, A., Brea, A., et al. (2012). Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. Gut, 61, 865-872.	Study design
Rea, G., Magee, A., & Loughrey, M. B. (2012). Streamlining the use of IHC in	Study design

identifying germline mismatch repair mutations in Lynch syndrome. Ulster Medical Journal, 81, 98-99.	
Ristimaki, A., Thiel, A., Heinonen, M., Kantonen, J., Lahtinen, L., Mecklin, J. P., et al. (2013). Immunohistochemical detection of BRAF V600E mutation in colorectal cancer and its use to identify Lynch syndrome patients. Virchows Archiv, 463 (2), 207-208.	Abstract only
Rodriguez-Soler, M., Perez-Carbonell, L., Guarinos, C., Zapater, P., Castillejo, A., Barbera, V. M., et al. (2013). Risk of cancer in cases of suspected lynch syndrome without germline mutation. Gastroenterology, 144, 926-932.e921; quiz e913-924.	Study design
Roncari, B., Pedroni, M., Maffei, S., Di Gregorio, C., Ponti, G., Scarselli, A., et al. (2007). Frequency of constitutional MSH6 mutations in a consecutive series of families with clinical suspicion of HNPCC. Clinical Genetics, 72, 230-237. doi: 10.1111/j.1399-0004.2007.00856.x	Study design
Salazar, R., Tian, S., Santos, C., Rosenberg, R., Nitsche, U., Mesker, W. E., et al. (2012). Development and validation of a genomic signature to identify colorectal cancer patients with microsatellite instability. Journal of Clinical Oncology. Conference, 30.	Abstract only
Serrano, M., Lage, P., Belga, S., Filipe, B., Francisco, I., Rodrigues, P., et al. (2012). Bethesda criteria for microsatellite instability testing: impact on the detection of new cases of Lynch syndrome. Familial Cancer, 11, 571-578.	Study design
Trano, G., & Sjursen, W. (2014). Molecular analyses of colrectal tumours may improve the identification of Lynch Syndrome related colorectal cancer. Colorectal Disease, 16, 92.	Study design
Wang, J., Luo, M. H., Zhang, Z. X., Zhang, P. D., Jiang, X. L., Ma, D. W., et al. (2007). Clinical and molecular analysis of hereditary non-polyposis colorectal cancer in Chinese colorectal cancer patients. World Journal of Gastroenterology, 13, 1612-1617.	Reference standard
Wang, J., Luo, M. H., Zhang, Z. X., Zhang, P. D., Jiang, X. L., Ma, D. W., et al. (2007). Clinical and molecular analysis of hereditary non-polyposis colorectal cancer in Chinese colorectal cancer patients. World Journal of Gastroenterology, 13, 1612-1617.	Reference standard
Warrier, S., Trainer, A., Lynch, A., Mitchell, C., Boussiotas, A., & Heriot, A. (2011). Preoperative diagnosis of lynch syndrome with DNA MMR immunohisto-chemistry on a diagnostic biopsy. Diseases of the Colon and Rectum, 54 (5), e23-e24.	Abstract only
You, J. F., Buhard, O., Ligtenberg, M. J. L., Kets, C. M., Niessen, R. C., Hofstra, R. M. W., et al. (2010). Tumours with loss of MSH6 expression are MSI-H when screened with a pentaplex of five mononucleotide repeats. British Journal of Cancer, 103, 1840-1845. doi: 10.1038/sj.bjc.6605988	Outcomes
Zahary, M. N., Kaur, G., Abu Hassan, M. R., Singh, H., Naik, V. R., & Ankathil, R. (2012). Germline mutation analysis of MLH1 and MSH2 in Malaysian Lynch syndrome patients. World Journal of Gastroenterology, 18, 814-820.	Study design

A4.2 Excluded full texts identified from systematic reviews

Reference	Primary reason for exclusion
Aaltonen, L. A., Salovaara, R., Kristo, P., Canzian, F., Hemminki, A., Peltomaki, P., et al. (1998). Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med, 338(21), 1481-1487. doi: 10.1056/NEJM199805213382101	Study design
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Reference	Primary reason for exclusion
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Vasen, H. F. A., Hendriks, Y., De Jong, A. E., Van Puijenbroek, M., Tops, C., Bröcker-Vriends, A. H. J. T., et al. (2004). Identification of HNPCC by molecular analysis of colorectal and endometrial tumors. Disease Markers, 20(4-5), 207-213.	Study design
Wagner, A., Barrows, A., Wijnen, J. T., Van Der Klift, H., Franken, P. F., Verkuijlen, P., et al. (2003). Molecular analysis of hereditary nonpolyposis colorectal cancer in the United States: High mutation detection rate among clinically selected families and characterization of an American founder genomic deletion of the MSH2 gene. American Journal of Human Genetics, 72(5), 1088-1100. doi: 10.1086/373963	Population
Yan, H. L., Hao, L. Q., Jin, H. Y., Xing, Q. H., Xue, G., Mei, Q., et al. (2008). Clinical features and mismatch repair genes analyses of Chinese suspected hereditary non-polypsis colorectal cancer: A cost-effective screening strategy proposal. Cancer Science, 99(4), 770-780. doi: 10.1111/j.1349-7006.2008.00737.x	Study design

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Truninger, K., Menigatti, M., Luz, J., Russell, A., Haider, R., Gebbers, J.O., et al. (2005). Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology, 128(5):1160-71.	Reference standard
Wagner, A., Barrows, A., Wijnen, J.Th., van der Klift, H., Franken, P.F., Verkuijlen, P., et al. (2003). Molecular Analysis of Hereditary Nonpolyposis Colorectal Cancer in the United States: High Mutation Detection Rate among Clinically Selected Families and Characterization of an American Founder Genomic Deletion of the MSH2 Gene. Am J Hum Genet, 72:1088–1100, 2003.	Population

Appendix 5. Summary of parameters in the health economic model

Parameter name		Base case value	Source
Population characteristics			
Max age		100	
Number of probands		34,025	ONS Cancer Registration Statistics for England (2014).[21]
Proportion of probands men		55%	ONS Cancer Registration Statistics for England (2006-2014).[4, 21, 94-96]
Number of relatives per proband		6	Assumption based on Snowsill et al. (2014)[4] and Barrow (2015)[84]
Proportion of relatives FDRs		42%	Snowsill et al. (2014)- published and unpublished data[4]
Proportion of relatives men		38%	Unpublished data reported in Snowsill et al. (2014)[4]
Prevalence of LS		2.8%	Hampel et al. (2008)[85]
Proportion of LS gene mutation	*MLH1*	32%	Palomaki et al (2009)[39]
	MSH2	39%	
	MSH6	14%	
	PMS2	15%	
Probability relative has LS if proband has LS		44%	Meta-analysis of published and unpublished data reported in Snowsill et al. (2014)[4]
Age on entry (probands)	General population	Distribution (Mean 73.0 years- model outcome)	Cancer registration statistics in England from 2006 to 2014.[21, 89-96]
	Lynch syndrome	Distribution (Mean 59.2 years- model outcome)	Estimated from the parametric colorectal cancer incidence function

Parameter name			Base case value	Source
Age on entry (relatives)	General population		Distribution (Mean 44.4 years-model outcome)	Mid-2014 population estimates for England and UK[97, 98]
	Lynch syndrome		Distribution (Mean 43.2 years-model outcome)	Without LS multiplied by CRC mortality free survival for LS. Truncated at 18 and 75 years[4]
Diagnostic parameters				
Test accuracy	MSI	Sensitivity	0.913 (0.426-0.993)	Barnetson et al. (2006)[52]
		Specificity	0.837 (0.638-0.937)	Poynter et al. (2008)[31] Southey et al. (2005)[54]
	IHC	Sensitivity	0.962 (0.694-0.996)	Limburg et al. (2011)[53]
		Specificity	0.884 (0.790-0.940)	Southey et al. (2005)[54]
	BRAF V600E	Sensitivity	0.96 (0.60-0.99)	Ladabaum et al. (2015)[3]
		Specificity	0.76 (0.60-0.87)	
	MLH1 promoter methylation	Sensitivity	0.94 (0.79-0.98)	Ladabaum et al. (2015)[3]
		Specificity	0.75 (0.59-0.86)	
	Diagnostic testing probands	Sensitivity	MLH1, MSH2, MSH6 0.90 PMS2 0.67	Dinh et al. (2011)[100]
		Specificity	0.997	Senter et al. (2008)[123]
	Predictive testing relatives	Sensitivity	1.00	Assumed (Snowsill et al. 2014)[4]
		Specificity	1.00	
Acceptance of diagnostic tests and genetic counselling	MSI	Proband	100%	Ramsey et al. 2003[76] confirmed by expert IMF in Snowsill et al. (2014)[4]
	IHC	Proband	100%	Assumed
	BRAF V600E	Proband	100%	Assumed

Parameter name			Base case value	Source
	MLH1 promoter hypermethylation	Proband	100%	Assumed
	Genetic test following counselling (proband)	Proband	90%	Ladabaum et al. 2011[78]
	Genetic counselling (proband)	Proband	92.5%	Clinical expert (IMF) range 90-95% in Snowsill et al. (2014)[4]
	Genetic test following counselling (relative)	Relative	77%	Manchester Familial colorectal cancer registry data reported in Barrow (2015)[84]
	Genetic counselling (relative)	Relative	78%	
Proportion of genetic tests in MSI strategies requiring IHC analysis			5%	Personal communication (Ottie O'Brien, Northern Molecular Genetics Service; Samantha Butler, West Midlands Regional Genetics Laboratory)
Proportion of probands who decline testing assumed to have LS			10%	Assumption based on Snowsill et al. (2014) results
Psychological disutility associated with testing for LS	Proband	Test declined, surgery not offered	0.04	Kuppermann et al. (2013)[157], Snowsill et al. (2014)[4]
		Test declined, accept TAHBSO	0.05 (women only)	
		Test declined, decline TAHBSO	0.11 (women only)	
		Test accepted, LS negative	0	
		Test accepted, LS positive, surgery not offered	0.02	
		Test accepted, LS positive, accept TAHBSO	0.03 (women only)	

Parameter name			Base case value	Source
		Test accepted, LS positive, decline TAHBSO	0.09 (women only)	
	Relative	Test declined, surgery not offered	0.04	
		Test declined, accept TAHBSO	0.08 (women only)	
		Test declined, decline TAHBSO	0.11 (women only)	
		Test accepted, LS negative	0	
		Test accepted, LS positive, surgery not offered	0.02	
		Test accepted, LS positive, accept TAHBSO	0.06 (women only)	
		Test accepted, LS positive, decline TAHBSO	0.09 (women only)	
Diagnostic costs	MSI		£202	UKGTN 2016[166]
	IHC		£210	UKGTN 2016[166] and personal communication (Dr Mark Arends, Department of Pathology, University of Cambridge; and IMF) from from Snowsill et al. (2014)[4]
	BRAF V600E		£119	UKGTN 2016, and Personal communication (Mr Michael Gandy, UCL-Advanced Diagnostics), East of Scotland Regional Genetic Service[168], All Wales Molecular Genetics Laboratory[169] from Snowsill et al. (2014)[4]

Parameter name		Base case value	Source
	MLH1 promoter methylation testing	£125	UKGTN 2016[166]
	Proband genetic test, all four genes	£1,276	UKGTN 2016[166]
	Proband genetic counselling	£63	The PSSRU (2014, 2015)[158, 167] and personal communication with Professor Mary Porteous (SE Scotland Genetic Service) from Snowsill et al. (2014)[4]
	Targeted genetic test for relatives (*MLH1*)	£166	UKGTN 2016[166]
	Targeted genetic test for relatives (*MSH2*)	£161	UKGTN 2016[166]
	Targeted genetic test for relatives (*MSH6*)	£161	UKGTN 2016[166]
	Targeted genetic test for relatives (*PMS2*)	£165	UKGTN 2016[166]
	Relative genetic counselling	£63	The PSSRU (2014,2015)[158, 167] and personal communication with Professor Mary Porteous (SE Scotland Genetic Service) from Snowsill et al. (2014)[4]
CRC parameters			
Acceptance of LS surveillance for CRC	Proband tested LS mutation positive	97%	Manchester Familial colorectal cancer registry[84]
	Proband LS assumed	70%	
	Relative tested LS mutation positive	97%	
	Relative LS assumed	70%	
Start age LS surveillance colonoscopy		25	Snowsill et al. (2014)[4]
End age LS surveillance colonoscopy		75	
HR associated with surveillance colonoscopy		0.387	Järvinen et al. (2000)[117]
Colonoscopy	Bleeding resulting in admission	0.0546%	Gavin et al. (2013)[160]

Parameter name			Base case value	Source
complication probability	Perforation		0.04%	
	Death		0.0083%	Cairns et al. (2010)[43]
CRC stage on diagnosis	Stage I		17.6%	National Cancer Intelligence Network, Cancer Survival in England by stage (2014)[124]
	Stage II		27.0%	
	Stage III		29.5%	
	Stage IV		25.9%	
CRC stage on diagnosis with LS surveillance colonoscopies	Stage I		68.6%	Mecklin et al. (2007).[125]
	Stage II		10.5%	
	Stage III		12.8%	
	Stage IV		8.1%	
Probability proband has colon cancer	With Lynch syndrome	Male	0.94	Dinh et al. (2011) online appendix[100]
		Female	0.94	
	Without Lynch syndrome	Male	0.63	ONS Cancer registration statistics, England 2013[96]
		Female	0.72	
Surgery for CRC	Colon	Segmental resection	96%	NHS Bowel Cancer Audit 2011[101]
		Subtotal colectomy	4%	
	Rectum	Anterior resection	98%	
		Proctocolectomy	2%	
Initial surgical state for probands	With Lynch syndrome		Segmental resection 90.7% Subtotal colectomy 3.3% Anterior resection 5.9% Proctocolectomy 0.1%	Calculated from above

Parameter name			Base case value	Source
	Without Lynch syndrome Men		Segmental resection 60.3% Subtotal colectomy 2.2% Anterior resection 36.8% Proctocolectomy 0.7%	
	Women		Segmental resection 69.6% Subtotal colectomy 2.6% Anterior resection 27.4% Proctocolectomy 0.5%	
Proportion of relatives who have previously has CRC	With Lynch syndrome	Men	None 96.8% Colon 2.98% Rectal 0.19%	Bonadona et al. (2011)[16] CRC incidence and CRC mortality statistics for England and Wales in 2010.[93, 103-105] Dinh et al. (2011)[100]
		Women	None 97.5% Colon 2.37% Rectal 0.15%	
	Without Lynch syndrome	Men	None 99.8% Colon 0.13% Rectal 0.09%	(UK) National Cancer Intelligence Network[102]
		Women	None 99.9% Colon 0.10% Rectal 0.04%	

Parameter name			Base case value	Source
Initial surgical state for relatives	With Lynch syndrome	Men	None 96.8% Segmental resection 2.88% Subtotal colectomy 0.11% Anterior resection 0.19% Proctocolectomy 0.00%	Calculated from above
		Women	None 97.5% Segmental resection 2.29% Subtotal colectomy 0.08% Anterior resection 0.15% Proctocolectomy 0.00%	
	Without Lynch syndrome	Men	None 99.8% Segmental resection 0.12% Subtotal colectomy 0.00% Anterior resection 0.09% Proctocolectomy 0.00%	

Parameter name			Base case value	Source
		Women	None 99.9% Segmental resection 0.09% Subtotal colectomy 0.00% Anterior resection 0.04% Proctocolectomy 0.00%	
Logistic model parameters for CRC incidence in individuals with Lynch syndrome	β_0	Men	0.464	Fit based on Bonadona et al. (2011)[16]
		Women	0.435	
	β_1	Men	0.107	
		Women	0.108	
	β_2	Men	55.5	
		Women	61.3	
Probability incident CRC is situated in the colon	With Lynch	No previous surgery	0.94	Dinh et al. (2011)[100]
		Segmental resection	0.94	
		Subtotal colectomy	0.00	Assumption
		Anterior resection	1.00	Assumption
		Proctocolectomy	N/A	Assumption
	Without Lynch	No previous surgery	Men 0.63 Women 0.72	ONS cancer registration statistics[93]
		Segmental resection	Men 0.63 Women 0.72	
		Subtotal colectomy	0.00	Assumption

Parameter name			Base case value	Source
		Anterior resection	1.00	Assumption
		Proctocolectomy	N/A	Assumption
Mortality rate from CRC (per 100,000 person years)	Years since diagnosis 0-1	Dukes' A	3,102	Calculated from (UK) National Cancer Intelligence Network[102]
		Dukes' B	8,709	
		Dukes' C	20,460	
		Dukes' D	96,729	
	1-2	Dukes' A	419	
		Dukes' B	5,000	
		Dukes' C	17,971	
		Dukes' D	67,733	
	2-3	Dukes' A	843	
		Dukes' B	4,761	
		Dukes' C	15,465	
		Dukes' D	51,116	
	3-4	Dukes' A	1,279	
		Dukes' B	4,000	
		Dukes' C	11,060	
		Dukes' D	32,857	
	4+	Dukes' A	1,400	
		Dukes' B	3,667	
		Dukes' C	9,068	
		Dukes' D	23,375	
Hazard ratios for CRC mortality age at diagnosis vs all ages	Under 70y	First year	0.599	Estimated using net survival statistics from the ONS,[111] Details in Appendix 6 of Snowsill et al. 2014.[4]
		1-4 years	0.972	
		After 4 years	1	

Parameter name			Base case value	Source
	70-79y	First year	0.956	
		1-4 years	0.966	
		After 4 years	1	
	80y and over	First year	1.797	
		1-4 years	1.116	
		After 4 years	1	
Hazard ratio for CRC survival with Lynch syndrome	Dukes' A and B		0.57	Lin et al. (1998)[112]
	Dukes' C and D		1	Barnetson et al. (2006)[52]
Disutilities associated with CRC	Dukes' A		0.00	Ramsey et al. 2000[132]
	Dukes' B		0.00	
	Dukes' C		0.00	
	Dukes' D		0.13	Mittmann et al. (2009)[133]
Disutility associated with CRC treatment (surgery, ostomies, chemotherapy)			0	Assumption based on literature review[142-147]
Disutility associated with colonoscopy			0	Assumption based on literature review[148]
Resource use colonoscopy	Lynch syndrome surveillance		Every 2 years from LS diagnosis (age 25-75 years)	Snowsill et al. (2014)[4]
	Post CRC surveillance		Every 5 years post CRC diagnosis	Snowsill et al. (2014)[4]
Unit cost colonoscopy			£585.80	NHS reference costs 2014–15[170] HRG codes FZ51Z, FZ52Z, FZ53Z
Cost of colonoscopy complication	Bleeding	Mild	£473	NHS reference costs 2014–15[170] HRG codes FZ38P
		Moderate	£1,138	FZ38J-FZ38L
		Severe	£4,394	FZ38G-FZ38H

Parameter name			Base case value	Source
	Perforation		£4,909	FZ77C-FZ77E
	Death		£4,909	Assumed same as perforation
CRC costs	Diagnosis		£1,022 (+£202 if Stage II for MSI testing)	Trueman et al. (2007)[116]
	Primary chemotherapy and radiotherapy	Colon	£14,494	
		Rectal Dukes' A	£1,049	
		Rectal Dukes' B	£4,206	
		Rectal Dukes' C	£9,504	
	Primary surgery	Segmental resection		NHS reference costs 2014–15[170] HRG codes FZ74-76, FZ50
		General population	£6,501	
		Lynch syndrome	£6,605	
		Subtotal colectomy with ileorectal anastomosis	£7,879	
		Rectal excision	£7,939	
		Proctocolectomy	£7,977	
	Follow up surveillance costs (excluding colonoscopy) per year	With Lynch syndrome	£229	Trueman et al.[116] NHS reference costs[170]
		Without Lynch syndrome	£230	
	Surgery and chemotherapy for recurrence	With Lynch syndrome	£12,333	Trueman et al.[116]
		Without Lynch syndrome	£12,236	

Parameter name			Base case value	Source
	Stoma care	With Lynch syndrome	£214	
		Without Lynch syndrome	£388	
	Palliative care	With Lynch syndrome	£9,907	
		Without Lynch syndrome	£9,665	
Endometrial cancer related parameters				
Gynaecological risk reduction for women with Lynch syndrome on entry	Age at diagnosis	0-34	No risk reduction 100% Surveillance 0.00% Prophylactic H-BSO 0.00%	Balmana et al. (2013),[118] Lorraine Cowley personal communication
		35-44	No risk reduction 20.0% Surveillance 60.0% Prophylactic H-BSO 20.0%	
		45-59	No risk reduction 16.7% Surveillance 45.8% Prophylactic H-BSO 37.5%	
		60-69	No risk reduction 0.00% Surveillance 14.3% Prophylactic H-BSO 85.7%	

Parameter name			Base case value	Source
		70+	No risk reduction 14.3% Surveillance 0.00% Prophylactic H-BSO 85.7%	
Lifetime EnCa risk in model	With Lynch syndrome		35%	Bonadona et al. (2011)[16]
	Without Lynch syndrome		0%	Assumption based on expected 1 in 41[113]
EnCa Survival		< 10 years since diagnosis	Piecewise constant rate of mortality for each year since diagnosis (77.5% alive at 10 years)	Uterine cancer survival statistics[114]
		>10 years mortality	0	Assumed
HR gynaecological surveillance on EnCa			0.898	Lewin et al. (2010)[106]
Disutility associated with EnCa			0.036	Nout et al. (2012)[149], Longworth et al. (2014)[154]
Length of tim EnCa disutility applied			1 year	Nout et al. (2012)[149]
Disutility associated with prophylactic H-BSO			0	Assumption based on disutility of EnCa treatment and long term disutility
Costs related to EnCa	Gynaecological surveillance		£473	NHS news story in 2011[171] NHS reference cost WF01A, MA36Z, MA25Z[170]
	Prophylactic H-BSO		£3,428	MA07E–MA07G, MA08A–MA08B NHS reference costs[170]
	EnCa	Surgery	£4,005	MA06A–MA06C NHS reference costs[170]
		Radiotherapy	£5,870	Havrilesky et al. (2009)[161]
		Adjuvant chemotherapy	£1,798	eMit database[172] SB14Z NHS reference costs (2015)[170]

Parameter name		Base case value	Source
Aspirin			
Offered and accept chemoprevention		80.4%	Burn et al. (2011)[28]
Proportion receive aspirin for 4 years		59.0%	
Incidence rate ratio CRC		0.37	
Incidence rate ratio EnCa		0.49	
Time aspirin effect lasts		10 years	
Daily dose		600mg	
Annual cost aspirin		£149	BNF (2016)[173]
Other parameters			
General mortality		Age dependent	England and Wales, 2008–2010,[115] adjusted by CRC mortality for England in 2010[103]
Discounting	Costs	3.5%	NICE reference case[178]
	QALYs	3.5%	

Appendix 6. Diagnostic meta-analysis code (Stata)

```
insheet using "MSI.csv", comma clear

insheet using "IHC.csv", comma clear

insheet using "MSI_L.csv", comma clear


gen long n1=tp+fn

gen long n0=fp+tn

gen long true1=tp

gen long true0=tn

gen long studyid= _n


reshape long n true, i(studyid) j(sens)


sort study sens

gen byte spec=1-sens


melogit true sens spec , nocons|| studyid: sens spec, ///

nocons cov(ind) binomial(n)


program define renamematrix, eclass

matrix mb = e(b)

matrix mv = e(V)

matrix colnames mb = logitse:_cons logitsp:_cons vlogitse:_cons vlogitsp:_cons
covlogits:_cons

matrix colnames mv = logitse:_cons logitsp:_cons vlogitse:_cons vlogitsp:_cons
covlogits:_cons

matrix rownames mv = logitse:_cons logitsp:_cons vlogitse:_cons vlogitsp:_cons
covlogits:_cons

ereturn post mb mv

end

renamematrix


_diparm logitse, label(Sensitivity) invlogit

_diparm logitsp, label(Specificity) invlogit
```