ERG erratum - Technology Assessment Report commissioned by the NIHR on behalf of the National Institute for Health and Clinical Excellence –Diagnostics Assessment Report

Title: *Testing strategies for Lynch syndrome in people with endometrial cancer*

Produced by: Warwick Evidence

Lead Author: Chris Stinton

Co-authors: Mary Jordan
Hannah Fraser
Peter Auguste
Rachel Court
Lena Alkhudairy
Jason Madan
Dimitris Grammatopoulos
Sian Taylor-Phillips

Correspondence to: Dr Sian Taylor-Phillips
Associate Professor
Division of Health Sciences
Warwick Medical School
[redacted]

Tel: [redacted]
Email: [redacted]

Date completed: *This addendum is current as of 5th June 2020*

Source of funding:

This report was commissioned by the NIHR Evidence Synthesis Programme as project number NIHR 129546.

Sian Taylor-Phillips is funded by an NIHR Career Development fellowship (CDF-2016-09-018).

Declared competing interests of the authors

None of the authors have any competing interests.

Acknowledgements

The authors would like to thank Professor Dimitris Grammatopoulos and Dr Mark Arends, for their expert clinical advice and to Professor Aileen Clarke and Dr Lazaros Andronis for

comments on the draft report. The authors would like to thank Kate Evans for helping with the coordinating of the report, and Sarah Abrahamson for formatting and checking the report.

Rider on responsibility for report

The views expressed in this report are those of the authors and not necessarily those of the NIHR Evidence Synthesis Programme. Any errors are the responsibility of the authors.

This report should be referenced as follows:

Testing strategies for Lynch syndrome in people with endometrial cancer. Diagnostic Assessment Report commissioned by the NIHR Evidence Synthesis Programme on behalf of the National Institute for Health and Care Excellence.

Contributions of authors

Rachel Court (Information specialist) developed the search strategy and undertook searches. Chris Stinton (Senior Research Fellow), Hannah Fraser (Research Associate), Lena Alkuhudairy (Senior Research Fellow), and James Keasley (Academic Foundation 2 doctor) conducted the clinical effectiveness systematic review, this included: screening and retrieving papers, assessing against the inclusion criteria, appraising the quality of papers and abstracting data from papers for synthesis. Chris Stinton (Senior Research Fellow) led the clinical effectiveness review. Mary Jordan (Research Fellow), Peter Auguste (Research Fellow) and Jason Madan (Professor in Health Economics) contributed to the cost-effectiveness review and undertook the health economic modelling. Dimitris Grammatopoulos (Professor of Molecular Medicine) provided clinical guidance and helped develop the model structures. Sian Taylor-Phillips (Associate Professor), led the project, and contributed to all stages for clinical and cost effectiveness. All authors were involved in writing draft and final versions of the report.

Academic and commercial in confidence information:

Please note that throughout the report academic in confidence (AIC) information is marked yellow and underlined and commercial in confidence (CIC) information is marked blue and underlined.

1. Three transitions permissible in the long term model were missing from the original version. They were: (1) No previous diagnosis of EC or CRC to diagnosed with CRC <1 year ago, (2) diagnosed with EC 1-2 years ago to diagnosed with EC and CRC, and (3) diagnosed with EC >10 years ago to diagnosed with EC >10 years ago. This error appeared on page 53 (figure 14).
2. Incorrect true positive and true negative values, and test accuracy estimates were reported for IHC with MLH1 methylation testing in relation to the paper by Lu et al.[14] These errors appeared on pages 116 – 117 (text and figure 25), and page 121 (Table 7) of the report.
3. Incorrect true negatives, and test accuracy estimates were reported for IHC, MSI, MLH1 methylation testing in relation to the paper by Salvador et al.[79] These errors appeared on pages 116 – 117 (text and figure 25), and page 121 (Table 7) of the report.
4. An incorrect reference was reported on page 119 of the report.
5. We incorrectly referred to Shin et al. as Shih et al.

3.3.2.2 Long-term outcomes model

We estimated the benefits of cascade testing by developing cohort state transition models that simulate the incidence and mortality associated with Lynch-related cancers. We use these models to predict the benefit of being identified with Lynch through cascade testing by simulating incidence and mortality with, and without, surveillance and risk reduction measures, which we assume are adopted once Lynch has been identified. The cohort that is modelled consists of a group of individuals identical in terms of age at which they were identified as having Lynch, sex, and previous Lynch cancer history (the model is repeated for a wide range of cohorts to provide the information needed for the decision tree model, this is described further in **Error! Reference source not found.** below).

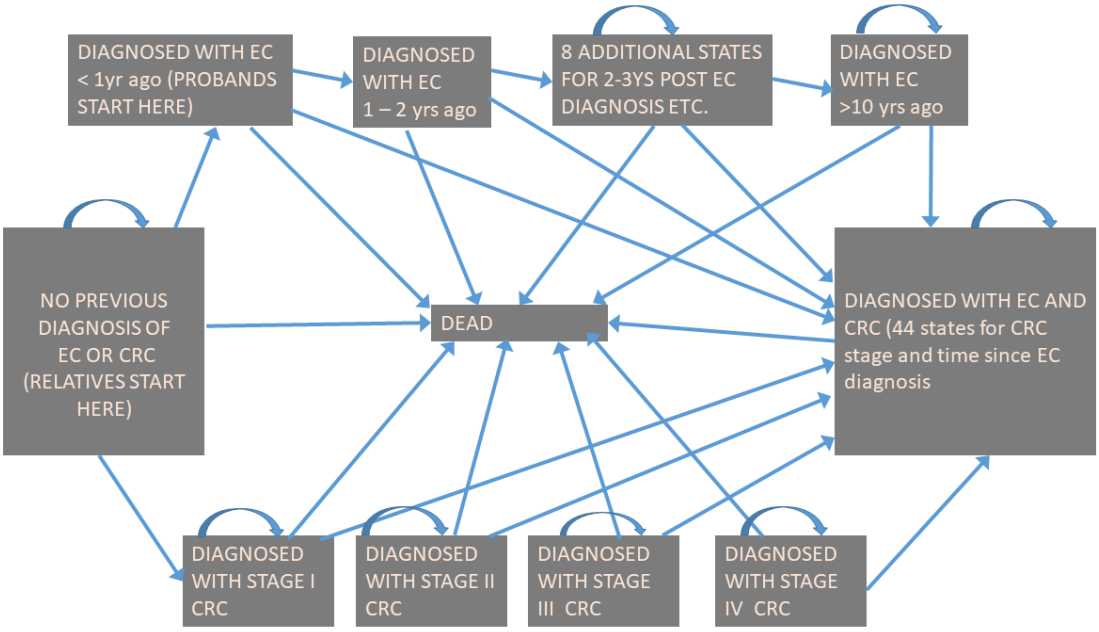

Figure 1: Overview of Long term model diagram

The model has five states – cancer free, CRC, EC, both CRC and EC, and dead. The EC state comprises 10 'tunnel states' reflecting time since incidence of EC. These are known as tunnel states because a person in this state must move to the next state in the sequence at the end of the cycle (unless they move to death). The cohort can be of any age from 0 to 100, male or female, and start in any state. The state for women who have both EC and CRC therefore has

(95% CI 79.1 - 100.0%), specificity 80.3% (95% CI 69.2 - 88.2%), positive predictive value 55.9% (95% CI 38.1 - 72.4%), negative predictive value 100.0% (95% CI 92.6 - 100.0%);[14] sensitivity 53.3% (95% CI 27.4 - 77.7%), specificity 76.5% (95% CI 64.4 - 85.6%), positive predictive value 33.3% (95% CI 16.4 - 55.3%), negative predictive value 88.1% (95% CI 76.5 - 94.7%).[78] These were similar to estimates in which variants of uncertain significance were consider to be germline negative.

Immunohistochemistry and microsatellite instability-based testing, with MLH1 promoter methylation testing

Four studies provided test accuracy data for immunohistochemistry and microsatellite instability-based testing, where a lack of expression on immunohistochemistry without MLH1 methylation or microsatellite instability:high (2 or more unstable markers) test was considered index test positive.[14, 54, 77, 79] The circumstances under which MLH1-PM was conducted varied in the studies. In two studies methylation testing was conducted in women who had tumours that were categorised as MSI-H or had IHC loss (MLH1 or MLH1/PMS2),[14, 79] in one study methylation testing was conducted in women who had IHC MLH1 loss only.[54] In the remaining paper, the circumstances under which MLH1-PM was conducted was not reported.[77] Three studies comprised selected samples of women,[14, 54, 79] and one study comprised an unselected sample of women.[77] One study excluded women over 50 years old,[14] one study excluded women with recurrent or synchronous cancers,[54] and one study included an unselected sample of women but did not report data on women with uninformative MMR results or without prior tumour testing.[79] Each study used a different panel of MSI markers. There were 85 true positives, 290 false positives, 475 true negatives, and 4 false negatives. Two studies reported the gene variants in LS cases.[14, 54] The most commonly affected gene was MSH2 (9/15 cases of LS, 60%), followed by MSH6 (4/15 cases of LS, 26.7%), MLH1 (2/15 cases of LS, 13.3%), and PMS2 (0/15 cases of LS, 0%). PMS2 was only assessed in 1 study.[54] In two studies, 25 variants of uncertain significance were identified (median = 12.5; 11 to 14 cases per study).[14, 54] One study did not report variants of uncertain significance.[79] In the remaining study, 25 variants of uncertain significance were identified but the study did not report whether the participants had had index testing.[77] Point estimates ranged from 90.5 – 100% for sensitivity, 6.6 – 92.3% for specificity, 18.3 - 56.3% for positive predictive values, and 75.0 – 100% for negative predictive values (see figure 25). In the study with an unselected sample of women, there were 19 true positives, 32 false positives, 312 true negatives, and 2 false negatives.[77] Comparing confidence intervals, there

was no statistically significant difference in sensitivity, specificity, positive predictive values, or negative predictive values between the studies with selected versus unselected samples

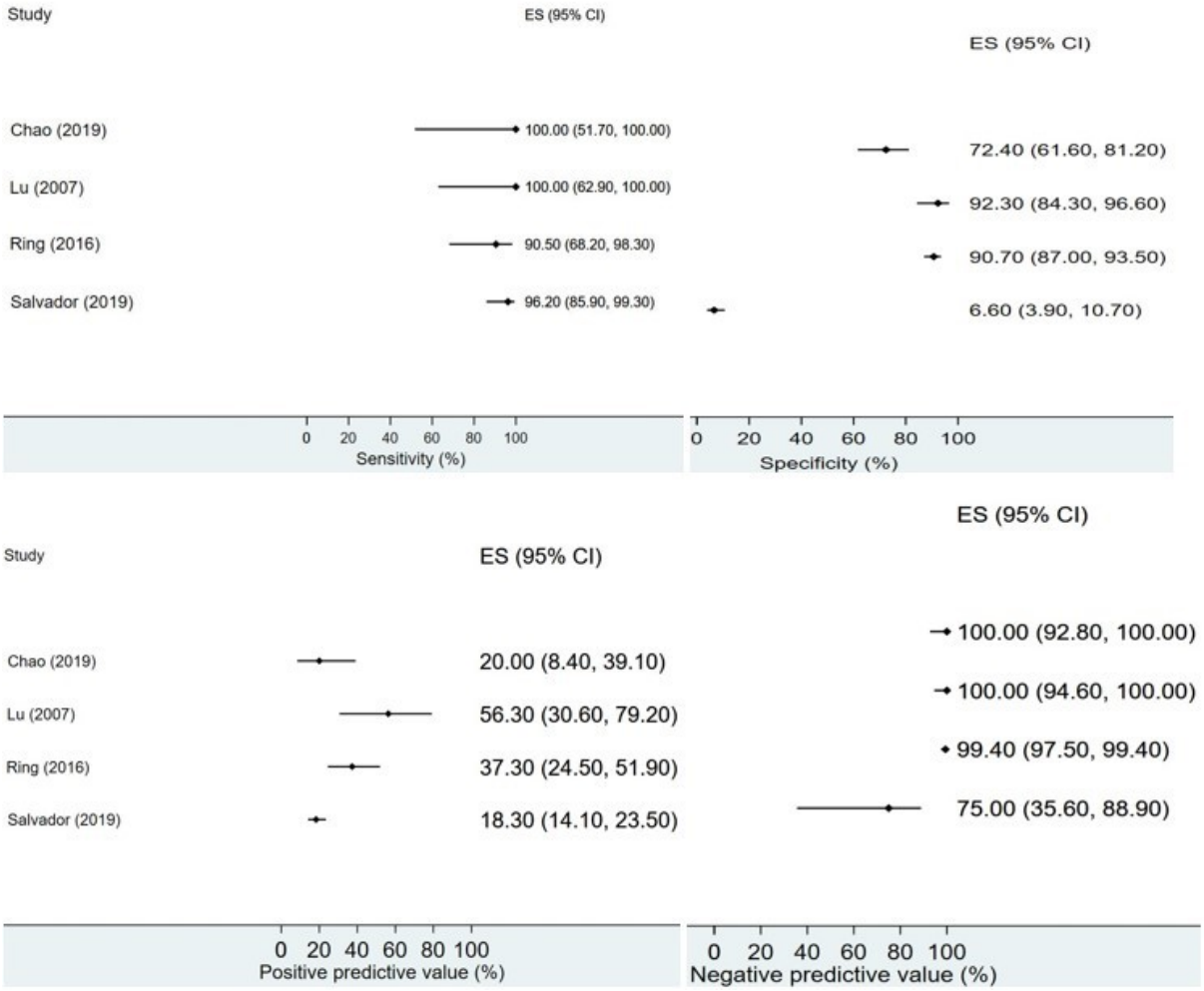

Figure 2: Sensitivity, specificity, positive predictive value, and negative predictive value of immunohistochemistry, microsatellite instability-based testing, and MLH1 promoter methylation testing for Lynch syndrome

Two studies reported the results of MLH1 promoter hypermethylation testing.[14, 54] Twelve out of 13 tumours (92.3%),[14] and 12 out of 15 tumours (80%) were hypermethylated.[54]

Data on test failures, indeterminate results, or lack of testing was reported in full for two studies.[14, 54] One study did not report any data on test failures, indeterminate results, or lack of testing,[77] and one study did not provide this data for MLH1 promoter hypermethylation testing.[79] Test failures were reported for 0 – 1% of tumours for immunohistochemistry (1 out of 567 tumours). No test failures were reported for microsatellelite instability-based testing or MLH1 promoter hypermethylation testing. No indeterminate results were reported of any of the three tests. Testing was not conducted in 0 – 8.1% for participants (9 out of 576 tumours) for immunohistochemistry, and 0.5 – 25.2% of participants (39 out of 372 tumours) for microsatellite instability-based testing due to insufficient tumour tissue (or unspecified reasons). There were no reported instances where MLH1 promoter hypermethylation testing could not be carried out.

Secondary analysis of test accuracy in which variants of uncertain significance were considered germline positive was possible for two studies.[14, 54] Estimates of test accuracy were as follows: Sensitivity 100.0% (95% CI 80.0 - 100.0%), specificity 86.3% (95% CI 75.8 - 92.9%), positive predictive value 66.7% (95% CI 47.1 - 82.1%), negative predictive value 100.0% (92.8 - 100.0%); [54] sensitivity 100.0% (80.0 - 100.0%), specificity 78.8% (67.9 - 86.8%), positive predictive value 54.1% (37.1 - 70.2%), negative predictive value 100.0% (92.8 - 100.0%).[14] These were similar to estimates in which variants of uncertain significance were consider to be germline negative with the except of positive predictive value for Chao et al,[54] which was higher when variants of uncertain significance were considered to be germline positive (66.7%, 95% CI 47.1 - 82.1% versus 20.0%, 95% CI 8.4% - 39.1%).

Concordance between immunohistochemistry and microsatellite instability-based testing

Twenty-three studies, including the unpublished PETALS study (personal communication, Ryan et al, University of Manchester, 11/12/2019), provided data on concordance between immunohistochemistry and microsatellite instability-based testing. [13, 14, 47, 50, 51, 54, 57-59, 63, 64, 67, 68, 71, 74-76, 78, 81-83, 87] Twenty studies provided complete concordance data (agreement/disagreement between IHC positive/negative and IHC negative), and 3 studies provided partial concordance data (IHC only conducted for MSI:H tumours,[83] MSI only conducted for women with IHC loss,[75] IHC only conducted for women with MSS results[13]). Full details of concordance are reported in **Error! Reference source not found.**. In the

studies providing complete concordance data, there was a high level of agreement between the results of the tests (median agreement = 91.8%, %, with the lowest level of agreement being 68.2% and the highest level of agreement being 100%) and a low level of disagreement (median disagreement = 9.8%, with the lowest level of disagreement being 0% and the highest level of adisgreement being 31.8%), median kappa 0.84 (range 0.32 – 0.97). Kappa values were calculated by the reviewers.

Few studies examined characteristics of discordant cases. Four studies reported that MLH1 promoter hypermethyation was common in discordant cases: 50% (1 out of 2 cases),[67] 75% (3 out of 4 cases),[68] 80% (4 out of 5 cases),[51] and 83% (10/12 cases).[82] 7 of the 23 concordance studies reported on the characteristics of discordant cases of MSI and IHC testing. [13, 14, 47, 51, 59, 68, 81] In 2 of these 6 studies it was possible to determine germline results for the discordant cases.[13, 51] Bruegl et al found 5.1% disagreement, with 7/197 discordant cases.[51] Of these 7 only 1 was found to have a germline mutation and this was in MSH6 variant. Likewise, Hampel et al found the only discordant case with a germline mutation was in the MSH6 variant.[13] Whereas, Lu et al found that of the 5 discordant cases, all were germline mutation negative.[14]

Across 3 studies, 20-57% (4/7, 1/5 and 2/6) of discordant results were due to MLH1 promoter hypermethylation, suggestive of epigenetic changes rather than Lynch syndrome. [14, 51, 68]

For one study, discordance was associated with the classification of MSI-L cases. When MSI-L cases were grouped with MSS cases, there were 2 discordant cases, whilst when MSI-H or MSI-L were grouped together and compared to MSS, there were no cases of discordance between MSI and IHC testing results.[47]

It was possible to calculate the average age for discordant cases in three studies.[13, 47, 81] In Anagnostopoulos et al, discordant cases (n=2) had a median age of 39.5 years, which was lower than the overall median in the sample of 48 years.[47] Whilst Shin et al and Hampel et al found no real difference in age between discordant cases and the whole sample. Shin et al found 2 discordant cases with a mean age of 55 years at diagnosis for EC cancer and 52.5 years for CRC compared to the overall sample mean age of 52.5 years for EC cancer and 54.5 years for CRC,[81] and Hamel et al found a mean age of 60.5 years in discordant cases compared to the overall mean of 60.9 years in the whole sample.[13]

There was 1 study which reported on the comorbidities of other cancers in discordant cases. All cases in the study had a history of both EC and CRC. They found 1 of the 2 discordant cases also had a history of bladder cancer. Likewise, this was the only study to discuss family history in relation to discordant cases, and noted that both cases met the Amsterdam II criteria. Further details on concordance are provided in **Error! Reference source not found.**.

Table 1. Complete test accuracy

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
MSI, IHC, and MLH1-PM											
Chao (2019)[54]	93	IHC (MLH1, MSH2, MSH6, PMS2) Negative staining of any of MMR protein MSI MSI-H:≥ 2 instable markers	NGS, Sanger sequencing	6	24	63	0	100.0% (51.7% - 100.0%)	72.4% (61.6% - 81.2%)	20.0% (8.4% - 39.1%)	100.0% (92.8% - 100.0%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
Lu (2007)[14]	100	IHC (MHL1, MSH2, MSH6) Loss of protein expression MSI MSI-H:≥ 2 instable markers	Sequencing, unspecified test for large deletions	9	7	84	0	100.0% (62.9% - 100.0%)	92.3% (84.3% - 96.6%)	56.3% (30.6% - 79.2%)	100.0% (94.6% - 100.0%)
Ring (2016)[77]	365	IHC (MLH1, MSH2, MSH6, PMS2) Complete absence of	MLPA, NGS	19	32	312	2	90.5% (68.2%, 98.3%)	90.7% (87.0%, 93.5%)	37.3% (24.5%, 51.9%)	99.4% (97.5%, 99.9%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
		MMR protein expression MSI MSI:H, but cut off not reported									
Salvador (2019)[79]	296	IHC (MLH1, MSH2, MSH6, PMS2) Cut off not reported MSI MSI-H:≥ 2 instable markers	MLPA, NGS	51	227	16	2	96.2% (85.9% - 99.3%)	6.6% (3.9% - 10.7%)	18.3% (14.1% - 23.5%)	75.0% (35.6 – 88.9%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
MSI only (MSI:H vs MSI:L/MSS)											
Berends (2003)[50]	57	MSI MSI-H ≥2 unstable markers	DGGE, sequencing, MLPA	4	16	36	1	80% (29.9-98.9%)	69.2% (54.7-80.9%)	20% (6.6-44.3%)	97.3% (84.2-99.9%)
Chao (2019)[54]	83	MSI MSI-H:≥ 2 instable markers	NGS, Sanger sequencing	4	8	71	0	100.0% (39.6% - 100.0%)	89.9% (80.5% - 95.2%)	33.3% (11.3% - 64.6%)	100.0% (93.6% - 100.0%)
Lu (2007)[14]	95	MSI MSI-H:≥ 2 instable markers	Sequencing, unspecified test for large deletions	8	17	70	0	100.0% (59.8% - 100.0%)	80.5% (70.3% - 87.9%)	32.0% (15.7% - 53.6%)	100.0% (93.5% - 100.0%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
Rubio (2016)[78]	83	MSI MSI-H, number of markers not specified	CSGE, sequencing, MLPA	5	16	55	7	41.7% (16.5-71.4%)	77.5% (65.7-86.2%)	23.8% (9.1-47.6%)	88.7% (77.5-95%)
MSI only (MSI:H/L vs MSS)											
Rubio (2016)[78]	83	MSI MSI-H/L, number of markers not specified	CSGE, sequencing, MLPA	5	17	54	7	41.7% (16.5-71.4%)	76.1% (64.2-85.1%)	22.7% (8.7-45.8%)	88.5% (77.2-94.9%)
IHC only											
Berends (2003)[50]	51	IHC (MLH1, MSH2, and MSH6) Absence of detectable	DGGE, sequencing, MLPA	5	18	28	0	100% (46.3-100%)	60.9% (45.4-74.5%)	21.7% (8.3-44.2%)	100% (85-100%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
		nuclear staining of cancer cells									
Chao (2019)[54]	102	IHC (MLH1, MSH2, MSH6, PMS2) Negative staining of any of MMR protein	NGS, Sanger sequencing	4	24	72	2	66.7% (24.1% - 94.0%)	75.0% (64.9% - 83.0%)	14.3% (4.7% - 33.6%)	97.3% (89.7% - 99.5%)
Lu (2007)[14]	99	IHC (MHL1, MSH2, MSH6)	Sequencing, unspecified test for large deletions	9	15	75	0	100.0% (62.9% - 100.0%)	83.3% (73.7% - 90.1%)	37.5% (19.5% - 59.2%)	100.0% (93.9% - 100.0%)

Study ID	Number tested	Index test and cut off	Reference standard	2x2 table				Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
				TP	FP	TN	FN				
		Loss of protein expression									
Rubio (2016)[78]	94	IHC Cut off not reported	CSGE, sequencing, MLPA	10	21	60	3	76.9% (46-93.8%)	74.1% (62.9-82.9%)	32.3% (17.3-51.5%)	95.2% (85.8-98.8%)
Tian (2019)[86]	165	IHC Cut off not reported	Sequencing/NGS, MLPA	41	115	8	1	97.6% (85.9 - 99.9)	6.5% (3.1 - 12.8%)	26.3% (19.7 - 34.0%)	88.9% (50.7 - 99.4%)

ACGH = Array Comparative Genomic Hybridisation; CC = colorectal cancer; CI = confidence interval; CSGE = conformation sensitive gel electrophoresis ; DGGE = denaturing gradient gel electrophoresis; EC = endometrial cancer ;MLH1-PM = MLH1 promoter methylation; MLPA =multiplex Ligation-dependent Probe Amplification; MMR = mismatch repair; NA = not applicable; NGS = next-generation sequencing; NPV = negative predictive value; PPV = positive predictive value; SSCV = single strand conformational variant