Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Clinical Excellence – Final Protocol

Title of project

Fluorouracil plasma monitoring: the My5-FU assay for guiding dose adjustment in patients receiving fluorouracil chemotherapy by continuous infusion

Name of External Assessment Group (EAG) and project lead

Produced by: Warwick Evidence

Lead author: Paul Sutcliffe

Co-authors: Martin Connock
Karoline Freeman
Ewen Cummins
Rachel Court
Sian Taylor-Phillips
Alexander Tsertsvadze
Ngianga-Bakwin Kandala
Tara Gurung
Ruth Pulikottil-Jacob
Mark Saunders
Aileen Clarke

Correspondence to: Dr Paul Sutcliffe
Associate Professor
Deputy Director for Warwick Evidence
Populations, Evidence and Technologies
Division of Health Sciences
Warwick Medical School
University of Warwick
Coventry CV4 7AL

Tel: 02476 150189

Fax: 02476 528375

Email: p.a.sutcliffe@warwick.ac.uk

Date completed: 19 December 2013

The views expressed in this protocol are those of the authors and not necessarily those of the NIHR HTA Programme. Any errors are the responsibility of the authors. The authors have no conflicts of interest.

1. Plain English Summary

5-fluorouracil (5-FU) is a chemotherapy medicine used to treat several cancers including those of the head and neck, pancreas, stomach and especially bowel (colorectal) cancer. 5-FU is usually given by continuous infusion into the blood circulation and is often accompanied by additional chemotherapies (known as adjuvant treatments). 5-FU is administered in a series of cycles usually over 3 to 6 months. After receiving 5-FU, it is cleared from patients' blood at rates that differ a lot from patient to patient, so the dose that reaches cancer cells varies considerably between individuals. As a result, some patients may receive doses that are too low to be fully effective, whereas others may experience toxicity because the circulating dose is too high. The My5-FU test kit is designed to measure the amount of 5-FU circulating in the blood using a small blood sample taken during the 5-FU infusion. Knowing the individual patient's level of 5-FU in the blood allows doctors to adjust the dose to be used at the next cycle of treatment so that it is appropriate for that individual. Giving a patient too much 5-FU may result in toxic effects which can reduce quality of life for the patient, whereas, giving a patient too little 5-FU might reduce the chances of the treatment working. The My5-FU assay is manufactured by Saladax Biomedical Inc. The My5-FU assay can be used with patients who have various types of cancer, however thus far most attention has been focussed on colorectal cancer, which is the third most common cancer in the UK, with around 40,000 new cases each year. The current report will allow NICE to make recommendations about how well the My5-FU assay works and whether the benefits are worth the cost of the tests for use in the NHS in England and Wales. The test allows a more tailored dosing of 5-FU which may lead to improved clinical outcomes and less side effects. The assessment will consider both clinical improvement in patients' symptoms and the cost of the test used to measure the amount of 5-FU.

2. Decision problem

The current report being undertaken for the NICE Diagnostics Assessment Programme examines the clinical and cost effectiveness of 5-FU plasma monitoring with the My5-FU assay for guiding dose adjustment in patients receiving 5-FU chemotherapy by continuous infusion.

2.1 5-Fluorouracil

5-Fluorouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is an antimetabolite of the pyrimidine analogue type, with a broad spectrum of activity against solid tumours (of the gastrointestinal tract, liver, pancreas, ovary, breast, brain, etc.), alone or in combination chemotherapy regimens.[1] The method of administration of 5-FU varies according to the type, location, and stage of cancer as well as the circumstances and preferences of the individual. 5-FU can be administered by infusion, injection, or orally as a pro-drug (e.g., capecitabine) and prescribed as either a single agent or in conjunction with other chemotherapy drugs.

Approximately 85% or more of administered 5-FU is inactivated and eliminated through the catabolic pathway; the remainder is metabolised through the anabolic pathway.[2] The enzyme dihydropyrimidine-dehydrogenase (DPD) has a major role in clearance of 5-FU and the rate of clearance (inactivation) varies considerably from patient to patient. 5-FU chemotherapy typically lasts 3 to 6 months and usually up to 12 cycles. Each cycle includes a period of 5-FU administration followed by a break to allow for recovery before the next cycle. Administration via continuous infusions usually lasts approximately 22 to 48 hours and requires patients to have a central venous access device such as a Hickman line or PICC line. Some patients have their 5-FU infusion via a portable pump which allows return to home during treatment.

Currently in most clinical practice in the UK the 5-FU dose administered is calculated according to patients' body surface area (BSA) and this dose remains unadjusted at subsequent cycles unless the patient experiences sufficient toxic effects to mandate dose reduction. Such dose reductions are guided by clinical judgment. The dose is not increased above an evidence-based (trial) maximum dose even if there is no toxicity.

2.2 Intervention technology

2.2.1 My5-FU

My5-FU (Saladax Biomedical, Inc., PA, USA; previously known as OnDose) is a nanoparticle immunoassay that measures levels of fluorouracil (5-FU) in plasma samples.[3] It is used with patients receiving 5-FU by continuous infusion to facilitate pharmacokinetic dose adjustment at the next cycle and drug monitoring to achieve an optimal plasma level of the drug. The assay uses two reagents: reagent 1 consists of a "5-FU conjugate" which is a 5-FU-like molecule linked to a long spacer arm; reagent 2 consists of antibodies covalently bound to nanoparticles, these antibodies are able to bind either 5-FU or the 5-FU conjugate. When reagents 1 and 2 are mixed the nanoparticles aggregate together. In the presence of free 5-FU some of the antibodies bind 5-FU rather than 5-FU conjugate, the amount of aggregation of nanoparticles is reduced and this alters the light absorbing properties of the mixture (that is 5-FU and "5-FU conjugate" compete for nanoparticle-bound antibodies). The light absorbance of the mixture is measured and can be compared against a calibrated standard curve in which light absorbance is graphed against known concentrations of free 5-FU in the mixture. In short, photometric detection (changes in absorbance) of nanoparticle aggregation allows determination of 5-FU concentration in plasma samples.[4] This assay can be performed on automated clinical chemistry analysers present in standard clinical laboratories. The assay requires a peripheral venous blood sample which is taken towards the end of each 5-FU infusion cycle using an EDTA or heparin tube.[5]

5-FU is amenable to therapeutic drug monitoring because it has a narrow therapeutic index, with doses below the therapeutic window potentially limiting treatment efficacy while doses above the window are more likely to cause side effects and toxicity. Commonly reported side effects of 5-FU chemotherapy include anaemia, thrombocytopenia, leukopenia, nausea/vomiting, diarrhoea, mucositis, and hand-foot syndrome,[6] all of which can be dose limiting when severe. Other consequences of 5-FU toxicity can include neuropathy, severe damage to organs, cardiotoxicity, neutropenia, sepsis and septic shock.[7] Patients with DPD deficiency are at significantly increased risk of developing severe and potentially fatal neutropenia, mucositis and diarrhoea when treated with 5-FU.[8, 9]

Results are reported in nanograms 5-FU/millilitre plasma and are converted to an area under the curve (AUC) value by multiplying the concentration of 5-FU in a steady state by the time of the infusion (in hours). This is then compared with a pre-defined optimal therapeutic range and the results, reported as mg·h/L, are used to guide the dose of 5-FU given in the next cycles. Outlier results greater than 50 mg·h/L are assumed to indicate that the blood sample has been taken too close to the infusion port and these results are disregarded. The My5-FU assay has been validated against liquid chromatography mass spectrometry (LC-MS)[4, 10] and high performance liquid chromatography (HPLC) laboratory techniques commonly used in pharmacokinetic studies.

When using the My5-FU assay in clinical practice, the initial dose of 5-FU is based on a patient's BSA. A blood sample is taken towards the end of the infusion cycle. For an infusion greater than 40 hours sampling is recommended at least 18 hours after starting infusion.[11] The sample should also be taken during a steady state period of the infusion which is usually about 4 hours before the end of the infusion using a non-battery operated device (which is commonly used in the UK). Depending on practice, it may require an additional visit by a district nurse or an additional outpatient attendance. Subsequent doses of 5-FU are calculated using the AUC result, according to a pre-determined dose adjustment algorithm. An example of a dose adjustment algorithm for patients with metastatic colorectal cancer recommends an optimal therapeutic range of 20–30 mg h/L with adjustments of no more than 30% of the dose for each infusion.[11] Patients typically require 3 or 4 pharmacokinetic-directed dose adjustments to reach an optimal therapeutic range.

2.3 Target conditions / indications

5-FU is commonly given to people with colorectal cancer, head and neck cancer, stomach cancer, pancreatic cancer and breast cancer. The general background and treatment pathways for each disease are summarised below.

2.3.1 Colorectal cancer

Background:

Colorectal cancer is the third most common cancer in the Western world and is the second most common cancer-related death in the combined male and female populations in the UK.[12] In 2010, there were 15,708 deaths from bowel cancer in the UK (62% from colon cancer, 38% from rectal cancer, including the anus), with 8,574 (55%) in men and 7,134 (45%) in women.[13-15] Around half of people diagnosed with colorectal cancer survive for at least five years after diagnosis.[16]

Following a diagnosis of colorectal cancer, patients who are more socioeconomically deprived are more likely to have both poorer cancer specific and overall survival.[17] Approximately 80% of patients with colorectal cancer undergo surgical treatment for the cancer with/without adjuvant radio- or chemotherapy (including 5-FU), but recurrence has been reported in 11% to 54% of patients.[18] More advanced cancers that have invaded other tissues or progressed to metastatic cancers tend to be treated with multiple chemotherapy drugs.

Advances in treatment and survival are likely to increase lifetime costs of managing colorectal cancer.[19] Cost-of-illness studies are key building blocks in economic evaluations of interventions and comparative effectiveness research. However, the methodological heterogeneity and lack of transparency of studies in this area have made it challenging to compare colorectal cancer costs between studies or over time.[19]

Care pathway:

The care pathway for patients with colorectal cancer is outlined in NICE Clinical Guideline 131 (CG131).[18] The following section will provide a summary of this information and that already detailed in the final scope provided by NICE.

Most patients with stage III colorectal cancer will be advised to have adjuvant therapy such as: capecitabine monotherapy or oxaliplatin in combination with 5-FU and folinic acid. Neoadjuvant chemoradiation may also be recommended prior to surgery for patients who are likely to have resectable tumours. The chemotherapy drugs most often used in the treatment of advanced colorectal cancer are 5-FU, capecitabine, raltitrexed, irinotecan, and oxaliplatin.[20]

NICE CG131[18] makes the following recommendations on the treatment of metastatic colorectal cancer: "When offering multiple chemotherapy drugs to patients with advanced and metastatic colorectal cancer, consider one of the following sequences of chemotherapy unless they are contraindicated:

- FOLFOX (folinic acid plus fluorouracil plus oxaliplatin) as first-line treatment then single agent irinotecan as second-line treatment or;
- FOLFOX as first-line treatment then FOLFIRI (folinic acid plus fluorouracil plus irinotecan) as second-line treatment or;
- XELOX (capecitabine plus oxaliplatin) as first-line treatment then FOLFIRI (folinic acid plus fluorouracil plus irinotecan) as second-line treatment."

Raltitrexed may be considered for patients who are intolerant to 5-FU and folinic acid or if these drugs are not suitable (e.g., patients who develop cardiotoxicity).

Oral therapy with fluoropyrimidines is recommended as an option for first line treatment of metastatic colorectal cancer. NICE technology appraisal 61 (TA61)[21] recommends capecitabine and tegafur with uracil as an alternative to intravenous 5-FU (tegafur with uracil is currently not being manufactured by any company and is therefore not available for use). When making a choice between oral and intravenous fluoropyrimidines clinicians should take into account contraindications and side effect profiles, the clinical condition and patient preferences. NICE Technology Appraisal 176 (TA176)[22] also recommends cetuximab in combination with FOLFOX or FOLFIRI chemotherapy for the first line treatment of metastatic colorectal cancer in people with KRAS wild-type tumours in whom:

- The primary tumour has been resected or is potentially operable;
- The metastatic disease is confined to the liver and is unresectable; and
- The patient is fit enough to undergo surgery to resect the primary colorectal tumour and to undergo liver surgery if the mestastases become resectable after treatment with cetuximab.

Recently, targeted agents for example cetuximab and panitumumab, and anti-vascular endothelial growth factor (VEGF) receptor agents, including anti-epidermal growth factor receptor (EGFR) agents such as bevacizumab have become available.[23] Although cetuximab and panitumumab appear to be clinically beneficial for KRAS wild-type patients compared with best supportive care, they are likely to represent poor value for money when judged by cost-effectiveness criteria currently used in the UK.[23] Because of this, cetuximab is only recommended under certain conditions by NICE.

Randomised controlled trials (RCTs) of anti-EGFR monoclonal antibodies (MAb) in patients with advanced colorectal cancer have reported inconsistent results.[24] For advanced colorectal cancer patients with KRAS wild-type, clear benefits of anti-EGFR MAbs in the third line and as first and second line, when used alongside infusional 5FU-based regimens have been identified. However, there appears to be no benefit for patients with KRAS mutations.[24]

5-FU administration:

The mode of administration (bolus or continuous infusion, or bolus followed by continuous infusion) of 5-FU varies considerably across the UK depending on facilities available in clinical settings. Administration using bolus injections has increasingly been replaced by continuous infusion regimens.[25] A comprehensive review by Hind et al. (2008)[26] evaluated three technologies for the management of advanced colorectal cancer: a) first-line irinotecan combination [with 5-fluorouracil (5-FU)] or second-line monotherapy; b) first- or second-line oxaliplatin combination (with 5-FU); and c) raltitrexed, where 5-FU is inappropriate. Hind et al. (2008)[26] identified the most common 5-FU regimens (see Table 1).

Table 1. 5-FU regimens description

Bolus schedules	
Mayo Clinic[27]	Monthly for 5 days with low-dose FA (5-FU 425 mg m^{-2}; FA 20 mg m^{-2})
Machover[28]	Monthly for 5 days with high-dose FA (5-FU 400 mg m^{-2}; FA 200 mg m^{-2} over 2h by infusion)
Roswell Park[29]	Weekly (5-FU 500 mg m–2; FA 500 mg m^{-2} over 2h by infusion)
Infusional schedules	
Lokich[30]	Protracted infusion (5-FU 300 mg m^{-2})
de Gramont[31] *	48-h both bolus and continuous infusion bimonthly (5-FU 400 mg m^{-2} bolus, 600 mg m^{-2} continuous infusion over 22 h, FA 200 mg m^{-2} over a 2-h infusion day 1 and 2 before 5-FU)
Modified de Gramont[32] *	48-h both bolus and continuous infusion bimonthly (5-FU 400 mg m^{-2} bolus, 2800 mg m^{-2} continuous infusion over 46 h; FA 175 mg m^{-2} over a 2-h infusion day 1 before 5-FU)
Grupo Espanol para el Tratamiento de Tumores Digestivos[33]	48-h infusion weekly (5-FU 3000 mg m^{-2})
Arbeitsgemeinschaft Internistische Onkologie[34]	24-h infusion weekly (5-FU 2600 mg m^{-2}; FA 500 mg m^{-2})
Chronomodulated delivery[35]	5-FU 700 mg m^{-2}; FA 300 mg m^{-2} per day, peak delivery rate at 04.00 h for 5 days

*These regimens are currently the two most used in the UK

Hind et al. (2008)[26] concluded that treatment for the management of advanced colorectal cancer with three active therapies appears most clinically effective and cost-effective. The authors encouraged future studies to analyse NHS routine data and undertake a meta-analysis using individual patient-level data to validate the optimal treatment sequence.

2.3.2 Head and neck, stomach and pancreatic cancers

The scope produced by NICE includes head and neck, stomach and pancreatic cancers. In Table 2 we provide a brief summary of the incidence and variety of 5-FU-based regimens itemised for these major cancers drawn from the NICE scope.

Table 2. Incidence and variety of 5-FU-based regimens

Cancer type	**Incidence of cancer**	**FU-based Regimen**
Head and neck cancer	• ≈16,000 people in UK are diagnosed each year • Mean national incidence rates are: ➢ 0.39 per 100,000 for nasopharyngeal cancer[36] ➢ 3.01 per 100,000 for laryngeal cancer[36] ➢ 3.02 per 100,000 for oral cancer[36] • Thyroid cancer has an estimated five year survival rate of 87%, and hypopharyngeal cancer is 26%[36]	• Nasal and sinus cancer: cisplatin, 5-FU, carboplatin, docetaxel, paclitaxel and gemcitabine • Nasopharyngeal cancer: cisplatin, 5-FU, docetaxel, paclitaxel and gemcitabine • Mouth and oropharyngeal cancer: cisplatin, 5-FU, carboplatin, bleomycin, methotrexate and docetaxel • Laryngeal cancer: cisplatin, 5-FU, carboplatin, taxol, capecitabine, and gemcitabine • Oesophageal cancer: epirubicin, 5-FU, capecitabine, cisplatin, oxaliplatin, taxol, irinotecan and vinorelbine
Stomach cancer	• 7,610 new cases of stomach cancer diagnosed in 2008[37] • Estimated five year survival rate of 18%[38]	• Epirubicin, cisplatin and fluorouracil • Epirubicin, oxaliplatin and fluorouracil • NICE Technology Appraisal 208 (TA208)[39] recommends trastuzumab in combination with cisplatin and capecitabine or 5-FU as an option for the treatment of HER2 positive metastatic adenocarcinoma of the stomach or gastro-oesophageal junction in people who have not received prior treatment for their metastatic disease and have tumours expressing high levels of HER2
Pancreatic cancer	• ≈8,500 people were diagnosed with pancreatic cancer in the UK in 2010[40] • 2.6% of cancer cases and 5% of all cancer deaths[40] • Estimated five year survival rate of less than 5%[41]	• Chemotherapy drugs that may be used to treat pancreatic cancer are 5-FU, capecitabine, and gemcitabine • NICE Technology Appraisal 25 (TA 25)[42]

3. Objectives

The overall objective of this report is to present the evidence on the clinical- and cost-effectiveness of the My5-FU assay for guiding dose adjustment in patients receiving 5-FU chemotherapy by continuous infusion (see desired outcomes in section 4.4.2).

The decision question taken from the NICE scope for this project is shown in the box below:

"What is the clinical and cost effectiveness of the My5-FU assay for the pharmacokinetic dose adjustment of continuous infusion 5-FU chemotherapy?"

In the current report we will:

A. Provide a review of the studies that examine the accuracy of the My5-FU assay when tested against gold standard methods of estimation of 5-FU or which develop a treatment algorithm based on plasma 5-FU measures. High performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) will be considered the gold standard for the purpose of assessing the accuracy of 5-FU plasma level measurements.

B. Systematically review the literature on the use of My5-FU to achieve adjusted dose regimen(s) to compare it with BSA-based dose estimation for patients receiving 5-FU-administered by continuous infusion. Variations in current BSA-based dose regimens will be considered where appropriate.

C. Systematically review the literature on the use of HPLC and/or LC-MS to achieve dose adjustment to compare it with BSA-based dose regimens for patients receiving 5-FU. This will be done for the purpose of performing a linked evidence analysis which must incorporate estimates of comparability of assay performance of My5-FU relative to the gold standards (HPLC, LC-MS) as outlined in A.

D. Provide an overview of systematic reviews of clinical outcomes in studies of 5-FU cancer therapies administered by continuous infusion in order to assess the generalizability of outcomes reported in the control arms of studies included in B and C above; outcomes of interest will include: incidence of side effects and 5-FU toxicity, treatment response rates, progression free survival, overall survival, and health related quality of life.

E. Identify evidence relevant to the costs of using My5-FU. Illustrative clinical pathways will be constructed; for this, we will use information provided by the manufacturer, advice from specialist committee members and other clinical experts, data collected from an identified UK

clinical laboratory and analysis of the published literature. We aim to collect information on the following:

a. Cost of My5-FU testing
b. Cost of delivering 5-FU by infusion
c. Cost of side effects and 5-FU toxicity and their associated treatment or hospitalisation

These will be considered from an NHS and Personal Social Services perspective.

Where possible, evidence will be synthesised to model the cost-effectiveness of My5-FU dose adjustment vs. BSA-based dose regimens in terms of cost per QALY with a lifetime horizon. Where the evidence does not directly support a complete "end-to-end" analysis from My5-FU through to overall survival, a linked evidence analysis may be undertaken. It is anticipated that for metastatic colorectal cancer (mCRC) a linked evidence approach to modelling will permit the impact of My5-FU upon dosing, and the impacts of this upon side effects and overall survival, to be estimated. As a consequence, the cost per QALY of My5-FU for mCRC will be estimated using a lifetime horizon. This estimate may none-the-less be subject to considerable uncertainty, both in terms of parameter values and model structure, and scenario and sensitivity analyses will be required.

Where the data is more limited (e.g., cancers other than colorectal), a truncated analysis may be undertaken. Further details are provided in sections 5.2.1 and 5.2.2. For instance, a truncated analysis might compare the treatment costs of infusion regimens with and without My5-FU, coupled with estimates of the cost and the quality of life impacts from any changes in side effect profiles. The underlying assumption to this analysis would be that pharmacokinetic dose adjustment is non-inferior to BSA dose adjustment in terms of progression free survival and overall survival.

Where a truncated analysis is undertaken, it will be augmented with threshold analyses that estimate what, if any, additional impacts My5-FU would be required to have upon progression free survival and/or overall survival for it to be cost-effective at conventional NICE willingness to pay thresholds.

4. Methods for assessing clinical effectiveness

Systematic review methods will follow the principles outlined in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care[43] and the NICE Diagnostic Assessment Programme manual.[44]

4.1 Data collection from a UK clinical laboratory

A questionnaire and semi-structured interview will be undertaken to gather information from a laboratory offering My5-FU testing. Questions will cover, but will not necessarily be limited to:

1. Are the proportions of samples from different cancers recorded? If so, what are the proportions?
2. Are the proportions of samples from different 5-FU therapies recorded? If so, what are the proportions?
3. Number of samples processed?
4. Costs of the test (fixed and variable costs)?
5. What are the staffing arrangements?
6. Turnaround time, including definition?
7. Any logistic / other issues related to the use of the test?

Information obtained from the face-to-face semi-structured interview and questionnaire will be used to provide information on My5-FU that has not been reported in studies included in the systematic reviews and overviews; this will inform the economic model. If any published reports on technical performance from NHS laboratories in England and Wales are identified by the searches, these will be summarised alongside the interview and questionnaire data.

4.2 Scoping searches

Initial scoping searches were undertaken to assess the volume and type of literature relating to the assessment questions. Search strategies were then developed which focused on records meeting the inclusion and exclusion criteria (see section 4.3.2). A draft search strategy was developed which focuses the searches on My5-FU/gold standard technologies, fluorouracil, pharmacokinetics and dose adjustment. Additional supplementary searches will be carried out as necessary. Searches for studies for cost and quality of life will be developed separately. All searches will be undertaken in January 2014.

4.3 Identification and selection of studies

4.3.1 Search strategies for clinical effectiveness

Scoping searches have been undertaken to inform the development of the search strategies (see above). An iterative procedure was used, with input from clinical advisors. A copy of the main draft search strategy that is likely to be used in the major databases is provided in Appendix 1. This strategy may be further refined and other appropriate concepts (e.g. cancer types) or limits (e.g. language, date, etc.) may be added. This search strategy developed for EMBASE will be adapted as appropriate for other databases. All retrieved papers will be screened for potential inclusion.

The search strategy will comprise the following main elements:

- Searching of electronic bibliographic databases
- Contact with experts in the field
- Scrutiny of references of included studies
- Screening of manufacturer's and other relevant organisations' websites for relevant publications

Bibliographic databases will include:
MEDLINE; MEDLINE In-Process & Other Non-Indexed Citations; EMBASE; Cochrane Library (including Cochrane Systematic Reviews, DARE, CENTRAL, NHS EED, and HTA databases); Science Citation Index and Conference Proceedings (Web of Science); NIHR Health Technology Assessment Programme; PROSPERO (International Prospective Register of Systematic Reviews).

The following trial databases will also be searched: Current Controlled Trials; ClinicalTrials.gov; UKCRN Portfolio Database; WHO International Clinical Trials Registry Platform.

Specific conference proceedings, to be selected with input from clinical experts and Specialist Committee Members, will be checked for the last five years.

The online resources of various health services research agencies and professional societies will be consulted via the Internet. These are likely to include:

- International Network of Agencies for Health Technology Assessment (INAHTA) Publication http://www.inahta.org/
- The Association of Cancer Physicians (ACP) http://www.cancerphysicians.org.uk/
- Royal College of Physicians: Oncology http://www.rcplondon.ac.uk/specialty/medical-oncology
- UK Oncology Nursing Society www.ukons.org/
- American Society of Clinical Oncology (ASCO) www.asco.org/
- Oncology Nursing Society http://www.ons.org/
- European Society for Medical Oncology (ESMO) www.esmo.org/
- European Oncology Nursing Society (EONS) http://www.cancernurse.eu/
- The BC Cancer Agency http://www.bccancer.bc.ca/
- The Association of Coloproctology of Great Britain and Ireland (ACPGBI) http://www.acpgbi.org.uk/
- British Society of Gastroenterology http://www.bsg.org.uk/

Citation searches of included studies will be undertaken using the Web of Science and Scopus citation search facilities. The reference lists of included studies and relevant review articles will be checked. Identified references will be downloaded in Endnote X7 software. Included papers will be checked for errata using PubMed.

4.3.2 Inclusion and exclusion of relevant studies

Inclusion of relevant studies to address objective A

Studies that compare the My5-FU assay with a gold standard, or that use My5-FU to develop a treatment algorithm, will be included. Plasma samples assayed come from people with cancer (i.e., colorectal cancer, head and neck cancer, stomach cancer, pancreatic cancer) receiving 5-FU chemotherapy by continuous infusion. All study designs will be considered for inclusion.

Inclusion criteria for studies to address objectives B and C

Study design:

All study designs will be considered for inclusion.

Healthcare setting:

Oncology services in secondary or tertiary care settings. Continuous infusion 5-FU can be delivered through oncology outpatient services, with patients returning home once an infusion has begun.

Population:

People receiving 5-FU chemotherapy by continuous infusion.

If evidence permits, this will include people with:

- Colorectal cancer
- Head and neck cancer
- Stomach cancer
- Pancreatic cancer

The use of My5-FU in patients receiving treatment in the adjuvant or metastatic setting will be considered separately if evidence permits. Population subgroups, in whom the use of the My5-FU assay may be particularly clinically and cost effective, will be considered separately if clinical outcomes allow. Such subgroups include:

- People with DPD deficiency
- People with impaired renal function

- People with impaired liver function
- People whose body surface area is outside the standard range for dosing 5-FU
- People with a less favourable performance status who may be undertreated in current practice

Intervention:
My5-FU assay.

To undertake a linked evidence analysis[45] evidence from studies using HPLC and LC-MS to adjust 5-FU cancer dosing will be evaluated.

Comparator:

- Current clinical practice for calculating and adjusting the dose of 5-FU is BSA dose adjustment
- Although not a comparator, HPLC and LC-MS will be considered the gold standard for the purpose of assessing the accuracy of 5-FU plasma level measurements
- No comparator

Outcomes:
Intermediate measures for consideration will include:

- Proportion of patients with 5-FU plasma levels in the optimal target range
- Area under the curve measurements
- Incidence of over and under dosing
- Frequency of dose adjustment
- Test failure rates

Clinical outcomes for consideration will include:

- Treatment response rates
- Progression free survival
- Overall survival
- Health related quality of life
- Incidence of side effects and 5-FU toxicity

<u>Exclusion criteria for studies to address objectives B and C</u>
Studies in patients receiving pro-drug 5-FU chemotherapy by oral therapy (e.g., capecitabine) and solely bolus delivery of 5-FU.

Inclusion criteria for studies to address objective D

Systematic reviews will be included if they satisfy the following criteria. If no relevant systematic reviews are identified, other study designs will be considered. In addition, the manufacturer's website will be screened for relevant publications.

Population studied:

- Populations corresponding to those investigated in the included studies that addressed objectives B and C

Intervention or comparator:

- 5-FU cancer treatments delivered by continuous infusion investigated in the studies included for objectives B and C
- No comparator

Outcomes:

- Treatment response rates
- Progression free survival
- Overall survival
- Health related quality of life
- Incidence of side effects and 5-FU toxicity

Inclusion criteria for studies to address objective E

All study designs will be considered for inclusion. Studies will be included that provide information on the following:

- Cost of My5-FU testing
- Cost of delivering 5-FU by infusion
- Cost of side effects and 5-FU toxicity and their associated treatment or hospitalisation costs
- Additional costs associated with changes to continuous infusion protocols

4.4 Review strategy

The general principles recommended in the PRISMA statement will be considered.[46] Records rejected at full text stage and reasons for exclusion will be documented. Two reviewers will independently screen the titles and abstracts of all records identified by the searches and discrepancies will be resolved through discussion. Disagreement will be resolved by retrieval of the full publication and consensus agreement. Full copies of all studies deemed potentially relevant, will be obtained and

two reviewers will independently assess these for inclusion; any disagreements will be resolved by consensus or discussion with a third reviewer.

4.5 Data extraction strategy

Data will be extracted by one reviewer, using a piloted, data extraction form. A second reviewer will check the extracted data and any disagreements will be resolved by consensus or discussion with a third reviewer. Examples of data extraction sheets for patient-based and diagnostic accuracy studies (My5-FU versus HPLC/LC-MS) are provided in Appendix 2.

4.6 Quality assessment strategy

Where appropriate, the quality of diagnostic accuracy studies will be assessed using QUADAS-2 (see Appendix 3).[47] As a broad range of study designs have been identified in the scoping searches, the use of a single checklist, in contrast to individual checklists for each study design, is considered appropriate. The Downs and Black checklist[48] will therefore be used to assess the quality of papers meeting the inclusion criteria (see Appendix 3). This 27-item checklist enables an assessment of randomised and non-randomised studies and provides both an overall score for study quality and a profile of scores not only for the quality of reporting, internal validity (bias and confounding) and power, but also for external validity. The results of the quality assessment will provide an overall description of the quality of the included studies and will provide a transparent method of recommendation for design of any future studies. Quality assessment will be undertaken by one reviewer and checked by a second reviewer, any disagreements will be resolved by a third reviewer through discussion.

The quality of primary economic evaluation studies (cost-effectiveness analysis) will be assessed using the CHEERS checklist[49] (see Appendix 3).

4.7 Methods of analysis/synthesis

4.7.1 Patient-based studies (objectives B and C)

Depending on the available evidence, analyses will be stratified according to cancer type, 5-FU delivery mode (for examples see Table 1) and cancer stage (e.g., metastatic).

Study, treatment, population, and outcome characteristics will be summarised and compared qualitatively and, where possible, quantitatively in text, graphically and in evidence tables. Pooling studies results by meta-analysis will be considered. Where meta-analysis is considered unsuitable for some or all of the data identified (e.g. due to the heterogeneity and/or small numbers of studies), we will employ a narrative synthesis. Typically, this will involve the use of text and tables to summarise

data. These will allow the reader to consider any outcomes in the light of differences in study designs and potential sources of bias for each of the studies being reviewed. Studies will be organised by research question addressed. A detailed commentary on the major methodological problems or biases that affected the studies will also be included, together with a description of how this may have affected the individual study results.

For objectives B and C we aim to identify studies that compare BSA-based dose regimens of 5-FU with continuous infusion in which measures of plasma 5-FU are not undertaken to inform dose changes with dose regimens in which dose adjustment is informed by the My5-FU assay results applied to a stated dose adjustment algorithm. These studies would best report the following outcomes: incidence and severity of side effects of 5-FU; overall survival and progression-free survival as stated in the present inclusion criteria. Such studies may be absent; therefore alternative study designs will be assessed. We will consider using a linked-evidence approach[45] in which studies report dose adjustment informed by plasma 5-FU measured by other methods (e.g. HPLC, LC-MS); this will require evidence of comparable performance of My5-FU with such assay methods.

In studies where My5-FU has been used but there is no comparator arm, or the comparator arm is a convenience sample (retrospective/historical population), outcomes will be listed and appraised. Outcomes reported for non-randomised comparator arms (i.e., historical controls) will be assessed for their representativeness in the light of information gained from systematic reviews (objective D). Relevant clinical outcomes from single arm studies may be considered for pooling should they be reported in sufficient detail and be considered relevant to the objectives.

Time to event outcomes:

We will request individual patient data (IPD) from authors of key included papers, to enable parameterisation of overall survival and progression-free survival implemented using standard parametric distributions. Goodness of fit to the observed data will be judged visually and according to information criteria (Akaike information criterion [AIC], Bayesian information criterion [BIC])). In the absence of IPD becoming available, we will digitise published Kaplan Meier (or competing risks) analyses using standard software (e.g. DigitizeIt software).[50] The digitised product will be used to construct curve fits using methods developed by Guyot et al. (2012)[51] or Hoyle and Henley (2011).[52]

Meta-analytic pooling:

If data allow, meta-analytic pooling of results from studies with both interventions (adjusted and unadjusted dose regimens) will be considered for incorporating summary time-to-event data.[18, 53] The decision to pool individual study results for time-to-event and binary outcomes will be based on

degree of similarity with respect to methodological and clinical characteristics of studies under consideration (e.g., design, population, comparator treatment, outcome). Should continuous outcomes be reported estimates of post-treatment mean difference (MD) will be pooled. For binary outcomes (except for rare events) pooling will use the DerSimonian and Laird random-effects model[54] The choice of this model is based on the assumption that some residual clinical and methodological diversity will exist across the pooled studies despite the similarities. Where necessary (zero events in one or both arms of a trial) a continuity correction will be applied. For binary outcomes with very low event rates < 1%, Peto odds ratios (ORs) will be pooled.

Single arm studies (observational and/or non-comparative studies) may be considered for pooling should they be relevant to the research objectives.

The degree of statistical heterogeneity across pooled studies will be assessed through visual inspection of the Forest plots, Labbe plots, calculation of Cochrans Q and tau-squared statistics for between study variance, and the I^2 statistics. Depending on the level of clinical and statistical heterogeneity subgroup and sensitivity analyses will be explored.

The extent of publication reporting bias will be examined by visual inspection, funnel plot asymmetry, and linear regression tests (Egger 1997,[55] for continuous outcomes, Harbord 2006,[56] and or Peters 2006,[57] for dichotomous outcomes), if a sufficient number of data points are available.

Indirect and mixed treatment comparison:
If there is a lack or insufficiency of evidence to allow a direct treatment comparison from head-to-head studies, and if time and data permit, in addition to a linked evidence approach we will consider undertaking a network or indirect comparison analysis for specific outcomes.[58-60]

4.7.2 Diagnostic accuracy studies (My5-FU versus HPLC/LC-MS) (Objective A)
The My5-FU assay delivers an estimate of plasma 5-FU concentration. For a study population this may potentially allow discrimination of study populations into categories: over-dosed, optimally-dosed and under-dosed. Should results from a gold standard also be available, a 2x2 table may be constructed allowing diagnostic accuracy to be estimated using standard statistics (e.g., sensitivity, specificity, positive and negative likelihood ratios, positive and negative predictive values).

Diagnostic accuracy studies (My5-FU versus HPLC/LC-MS) are considered to be those where patient samples are assayed for 5-FU concentration but patient outcomes may not be reported. Those studies that aim to test the internal and/or external validity of the My5-FU assay will be identified and their

findings will be summarised and appraised. Studies that do not report test failure rates will be noted; where available, test failure rates will be tabulated.

4.8 Face-to-face semi-structured interview and questionnaire

Information will be extracted from the interview and questionnaire conducted with the relevant laboratory. A narrative summary of the questionnaire data will be provided and may be used to populate the economic model parameters. If gaining this information is not feasible within the timeframe, expert opinion from Specialist Committee Members and other clinical experts will be sought and appropriately cited.

5. Report methods for synthesising evidence of cost-effectiveness

5.1 Identifying and reviewing published cost-effectiveness studies

Published cost-effectiveness studies will be reviewed. All papers which present findings on the cost and outcomes of pharmacokinetic dose adjustment will be reviewed in detail, with a particular emphasis upon those which assess pharmacokinetic dosing compared to BSA dosing.

5.1.1 Search strategy

A comprehensive search of the literature for published economic evaluations, utility studies and cost studies will be performed. The search strategy used will be based on the strategy developed for the clinical effectiveness review (see Appendix 1) and is likely to focus on fluorouracil and dose adjustment, combined with a methodological study design filter where appropriate.

Databases will include:

- MEDLINE (Ovid)
- MEDLINE In-Process Citations and Daily Update (Ovid)
- EMBASE (Ovid)
- NHS Economic Evaluation Database (NHS EED) (Cochrane Library)
- Science Citation Index (Web of Knowledge)
- Research Papers in Economics (REPEC)

Additional searches will be performed where necessary to identify other relevant information to support the development of an economic model for this project. For example, a tightly focussed search for fluorouracil, relevant cancers and cost-effectiveness may be required to identify comparator costs, etcetera. Clinical trials as well as modelling studies and cohort studies will be considered.

5.2 Evaluation of costs, quality of life and cost-effectiveness

5.2.1 Model structure

Where data allows, the preferred approach will be to model the impact of pharmacokinetic dose adjustment using My5-FU assay compared to BSA dosing, using the clinical outcomes specified in section 4.3.2 above and with a lifetime horizon. In the absence of such evidence a linked evidence approach will be adopted, linking My5-FU dose adjustment to other pharmacokinetic dose adjustment studies within the literature. It may assume equivalence between the My5-FU assay and other pharmacokinetic measures of plasma 5-FU (i.e., HPLC and LC-MS) if this appears a reasonable assumption in the light of the clinical review. Model inputs may utilise indirect treatment comparison results or network meta-analysis results to derive estimates of the clinical outcomes for the chemotherapy regimens relevant to current UK clinical practise. It is anticipated that this will be possible for metastatic colorectal cancer, as outlined in more detail in section 5.2.2.

While it is desirable to try to link evidence through to final survival outcomes, it should be recognised that due to data limitations this may be impossible for some cancers. Where this applies, the assessment will still endeavour to estimate the impact of My5-FU dose adjustment upon test costs, treatment costs, side effect costs and the quality of life impacts of side effects. This truncated analysis will be augmented by threshold analyses that estimate what, if any, additional impacts My5-FU would be required to have upon progression free survival and/or overall survival for it to be cost-effective at conventional NICE willingness to pay thresholds. The estimates of the additional survival will be reported in terms of the absolute additional time required, with this being compared with estimates of the relevant current mean survival. It will also be reported in the same metric as that used for the estimate of the impact of pharmacokinetic dose adjustment upon overall survival in the mCRC modelling, in order to facilitate a comparison across clinical areas; e.g., as a relative risk or as a hazard ratio.

Necessary choices and definitions regarding the structure of the model will depend on the findings from the literature review and consultation with clinical experts.

5.2.2 Issues relevant to analyses

During scoping no end-to-end studies of the My5-FU assay were identified. Evidence was found relating to: the validation of the My5-FU assay with LC-MS, the impact of 5-FU plasma levels on toxicity, pharmacokinetic variability of 5-FU when BSA dosing is used, and the impact of pharmacokinetic dose adjustment of 5-FU on survival. Studies comparing pharmacokinetic dosing with BSA dosing in mCRC were found that reported average 5-FU weekly doses, adverse event rates, progression free survival and overall survival with varying degrees of completeness (Capitain 2012;[61]

Gamelin 2008[62]). These, together with other papers that may be identified during the literature searches, may provide sufficient information to enable estimation of the various clinical outcomes for mCRC. The papers' authors will also be approached for the information about the outcomes that were ambiguously, partially or not reported for one or both arms.

Where clinical outcome estimates can be arrived at for My5-FU informed dose adjustment and BSA dosing, these will be the preferred basis of the modelling. The main model structure will be developed to favour these elements over those that may be drawn from a linked evidence approach or from expert opinion. This does not preclude more speculative model structures also being developed.

One way sensitivity analyses will be performed for all key parameters, and for parameters in the models which are based on expert opinion or lie within any more speculative linked evidence modelling. The appropriate model structure may also be subject to some uncertainty. Probabilistic modelling will be performed using parameter distributions instead of fixed values. It may be necessary to perform a number of probabilistic modelling exercises, given the uncertainty around parameter estimates that are based upon expert opinion and the uncertainty around the most appropriate model structure.

Decision uncertainty regarding mutually exclusive alternatives will be reflected using cost-effectiveness planes and cost-effectiveness acceptability curves or frontiers.

Longer term costs and consequences will be discounted using the UK discount rates of 3.5% for both costs and effects.

5.2.3 Health outcomes

Utility values, based on literature or other sources, will be incorporated in the economic model. QALYs will be calculated from the economic modelling.

5.2.4 Costs

Data for the cost analyses will be drawn from routine NHS sources (e.g. NHS reference costs, Personal Social Services Research Unit [PSSRU], British National Formulary [BNF]), discussions with individual hospitals and with the manufacturer.

Costs for consideration will include:

- Cost of My5-FU testing
- Cost of delivering 5-FU by infusion

- Cost of side effects and 5-FU toxicity and their associated treatment or hospitalisation costs
- Additional costs associated with changes to continuous infusion protocols

Other costs for consideration may include:

- Cost of 2nd line therapies
- Palliative care and end of life costs

5.3 Cost and resource use

Resource use will be estimated in line with the DAP programme manual:

- The perspective will be that of the NHS and PSS
- The cost of the My5-FU assay will be requested from the manufacturer on the basis of this being nationally and publicly available, with additional confirmation of this sought from a UK laboratory currently using My5-FU
- The base case will use list prices for the chemotherapy regimens, but as in the modelling for CG131 the impact of discounted prices available to the NHS may also be explored
- The above two bullets may be augmented with advice from the UK NHS centre currently using the My5-FU assay and possibly bodies such as the NHS Purchasing and Supply Agency (PASA)
- The effect of My5-FU upon resource use in terms of physical units will be presented separately and then coupled with unit costs

6. Handling of information from manufacturers

All data submitted by the manufacturers/sponsors will only be considered if received by the External Assessment Group before 20/03/2014. Data arriving after this date will not be considered. Any data that meets the inclusion criteria stated will be extracted and quality assessed as stated in the methods section of this protocol.

Any ‘commercial in confidence’ data provided by manufacturers, and specified as such, will be highlighted in blue and underlined in the assessment report (followed by company name in parentheses). Any ‘academic in confidence’ data provided by manufacturers, and specified as such, will be highlighted in yellow and underlined in the assessment report. All confidential data used in the cost-effectiveness models will also be highlighted.

7. Competing interests of authors and advisors

None of the authors have any competing interests.

8. Timetable/milestones

Draft assessment protocol	**27/11/2013**
Final protocol	**19/12/2013**
Progress report	**20/03/2014**
Draft assessment report	**21/05/2014**
Final assessment report	**18/06/2014**

9. Team members' contributions

Warwick Evidence is an External Assessment Group located within Warwick Medical School. Warwick Evidence brings together experts in clinical and cost effectiveness reviewing, medical statistics, health economics and modelling. The team planned for the work include:

Lead:	Dr Paul Sutcliffe
Title:	Associate Professor
Address:	Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel:	02476 574505
Email:	p.a.sutcliffe@warwick.ac.uk
Contribution:	Co-ordinate review process, protocol development, assessment for eligibility, quality assessment of trials, data extraction, data entry, data analysis, and report writing

Name:	Dr Martin Connock
Title:	Senior Research Fellow
Address:	Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel:	02476 574940
Email:	M.Connock@warwick.ac.uk
Contribution:	Protocol development, assessment for eligibility, quality assessment of trials, data analysis, statistical modelling, and report writing

Name:	Mrs Karoline Freeman
Title:	Research Fellow
Address:	Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel:	02476 574026

Email: K.Freeman@warwick.ac.uk
Contribution: Assessment for eligibility, quality assessment of trials, data extraction, data entry, and report writing

Name: Dr Ewen Cummins
Title: Health economist
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 01413 373187
Email: ecummins@mcmdc.com
Contribution: Protocol development, health economics modeller, data analysis, and report writing

Name: Ms Rachel Court
Title: Information Specialist
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 522427
Email: R.A.Court@warwick.ac.uk
Contribution: Protocol development, develop search strategy and undertake the electronic literature searches

Name: Dr Sian Taylor-Phillips
Title: Senior Research Fellow
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 575882
Email: S.Taylor-Phillips@warwick.ac.uk
Contribution: Protocol development, data analysis, and report writing

Name: Dr Alexander Tsertsvadze
Title: Senior Research Fellow
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 574505
Email: a_tsertsvadze@hotmail.com

Contribution: Assessment for eligibility, quality assessment of trials, data extraction, data analysis, and report writing

Name: Dr Ngianga-Bakwin Kandala
Title: Principal Research Fellow
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 575054
Email: N-B.Kandala@warwick.ac.uk
Contribution: Data analysis and statistical modelling

Name: Dr Tara Gurung
Title: Research Fellow
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 150711
Email: t.gurung@warwick.ac.uk
Contribution: Assessment for eligibility, data extraction, data entry, and report writing

Name: Ms Ruth Pulikottil-Jacob
Title: Research Fellow Health Economics
Address: Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 151902
Email: R.Jacob@warwick.ac.uk
Contribution: Health economics modeller, assessment for eligibility and data extraction

Name: Professor Aileen Clarke
Title: Director of Warwick Evidence
Address: Warwick Evidence, Populations, Evidence and Technologies, Division of Health Sciences, Warwick Medical School, University of Warwick, Coventry CV4 7AL
Tel: 02476 150189
Email: Aileen.Clarke@warwick.ac.uk
Contribution: Co-ordinate review process, protocol development, synthesis of findings and report writing

9.1 Expert advisors

Name:	Dr Mark Saunders
Title:	Consultant Clinical Oncologist
Address:	The Christie, Wilmslow Road, Manchester M20 4BX
Tel:	07590 040194
Email:	mark.saunders@christie.nhs.uk
Contribution:	Provide expert clinical advice on cancer and care pathways

Name:	Dr Michael Chappell
Title:	Senior Lecturer
Address:	Warwick Engineering in Biomedicine, University of Warwick, Coventry CV4 7AL
Tel:	02476 524309
Email:	M.J.Chappell@warwick.ac.uk
Contribution:	Provide expert advice on biomedicine

10. References

1. Arias J. Novel Strategies to Improve the Anticancer Action of 5-Fluorouracil by Using Drug Delivery Systems. *Molecules*. 2008;**13**(10):2340-69.

2. BlueCross BlueShield of Alabama. Laboratory Testing to Allow Area Under the Curve (AUC) Targeted 5-Fluorouracil (5-FU) Dosing for Patients Administered 5-FU for Cancer. 2013 April 2013 [cited 15/11/2013]; Available from: https://www.bcbsal.org/providers/policies/final/253.pdf.

3. Salamone SJ, Li Y, Courtney J, Harney R, Lundell G, Stocker D. 5-Fluorouracil determination in plasma by rapid nanoparticle immunoassay; Highlights from: 5-Fluorouracil Drug Management Pharmacokinetics and Pharmacogenomics Workshop; Orlando, Florida; January 2007. *Clin Colorectal Cancer*. 2007;**6**(6):419-20.

4. Beumer JH, Boisdron-Celle M, Clarke W, Courtney JB, Egorin MJ, Gamelin E, *et al.* Multicenter evaluation of a novel nanoparticle immunoassay for 5-fluorouracil on the Olympus AU400 analyzer. *Therapeutic drug monitoring*. 2009;**31**(6):688-94.

5. Saladax Biomedical. 5-Fluorouracil (My5-FU™) Assay [package insert]. 2011 [cited 19/11/2013]; Available from: http://www.saladax.com/wp-content/uploads/2012/07/My5-FU-Assay-Package-Insert-rev06.pdf.

6. Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. Meta-analysis Group In Cancer. *J Clin Oncol*. 1998;**16**(1):301-8.

7. Hale JP. Costs and consequences of different chemotherapy regimens in metastatic colorectal cancer. *British Journal of Cancer*. 2002;**86**(11):1684-90.

8. Lee A, Ezzeldin H, Fourie J, Diasio R. Dihydropyrimidine dehydrogenase deficiency: impact of pharmacogenetics on 5-fluorouracil therapy. *Clinical advances in hematology & oncology : H&O*. 2004;**2**(8):527-32.

9. Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, *et al.* Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. *J Clin Oncol*. 2008;**26**(13):2131-8.

10. Buchel B, Rhyn P, Schurch S, Buhr C, Amstutz U, Largiader CR. LC-MS/MS method for simultaneous analysis of uracil, 5,6-dihydrouracil, 5-fluorouracil and 5-fluoro-5,6-dihydrouracil in human plasma for therapeutic drug monitoring and toxicity prediction in cancer patients. *Biomedical Chromatography*. 2013;**27**(1):7-16.

11. Kaldate RR, Haregewoin A, Grier CE, Hamilton SA, McLeod HL. Modeling the 5-fluorouracil area under the curve versus dose relationship to develop a pharmacokinetic dosing algorithm for colorectal cancer patients receiving FOLFOX6. *The oncologist*. 2012;**17**(3):296-302.

12. Mansouri D, McMillan DC, Grant Y, Crighton EM, Horgan PG. The impact of age, sex and socioeconomic deprivation on outcomes in a colorectal cancer screening programme. *PloS one*. 2013;**8**(6):e66063.

13. Office for National Statistics (ONS). Mortality Statistics: Deaths registered in 2010, England and Wales. London: ONS, 2011.

14. General Register Office for Scotland. Deaths Time Series Data: Deaths, by sex, Scotland, 1855 to 2012. 2013 [cited 19/11/2013]; Available from: http://www.gro-scotland.gov.uk/statistics/theme/vital-events/deaths/time-series.html.

15. Northern Ireland Statistics and Research Agency (NISRA). Registrar General Annual Report 2011. Belfast: NISRA, 2012.

16. Cancer Research UK. Bowel cancer survival statistics. 2012 [cited 14/11/13]; Available from: www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/survival/.

17. Hole DJ, McArdle CS. Impact of socioeconomic deprivation on outcome after surgery for colorectal cancer. *British Journal of Surgery*. 2002;**89**(5):586-90.

18. National Institute for Health and Care Excellence. Colorectal cancer: the diagnosis and management of colorectal cancer. CG131. 2011 [cited 14/11/2013]; Available from: http://www.nice.org.uk/guidance/CG131.

19. AJ OC, Hanly P, Skally M, O'Neill C, Fitzpatrick P, Kapur K, *et al*. Cost comparisons and methodological heterogeneity in cost-of-illness studies: the example of colorectal cancer. *Medical care*. 2013;**51**(4):339-50.

20. Cancer Research UK. Side effects of bowel cancer chemotherapy. 2013 [cited 19/11/13]; Available from: http://www.cancerresearchuk.org/cancer-help/type/bowel-cancer/treatment/chemotherapy/side-effects-of-bowel-cancer-chemotherapy.

21. National Institute for Health and Care Excellence. Capecitabine and tegafur uracil for metastatic colorectal cancer. TA61. 2003 [cited 19/11/2013]; Available from: http://www.nice.org.uk/guidance/TA61.

22. National Institute for Health and Care Excellence. Cetuximab for the first line treatment of metastatic colorectal cancer. TA176. 2009 [cited 19/11/2013]; Available from: http://www.nice.org.uk/guidance/TA176.

23. Hoyle M, Crathorne L, Peters J, Jones-Hughes T, Cooper C, Napier M, *et al*. The clinical effectiveness and cost-effectiveness of cetuximab (mono- or combination chemotherapy), bevacizumab (combination with non-oxaliplatin chemotherapy) and panitumumab (monotherapy) for the treatment of metastatic colorectal cancer after first-line chemotherapy (review of technology appraisal No.150 and part review of technology appraisal No. 118): a systematic review and economic model. *Health technology assessment (Winchester, England)*. 2013;**17**(14):1-237.

24. Vale CL, Tierney JF, Fisher D, Adams RA, Kaplan R, Maughan TS, *et al.* Does anti-EGFR therapy improve outcome in advanced colorectal cancer? A systematic review and meta-analysis. *Cancer treatment reviews*. 2012;**38**(6):618-25.

25. Saif MW, Choma A, Salamone SJ, Chu E. Pharmacokinetically guided dose adjustment of 5-fluorouracil: A rational approach to improving therapeutic outcomes. *Journal of the National Cancer Institute*. 2009;**101**(22):1543-52.

26. Hind D, Tappenden P, Tumur I, Eggington S, Sutcliffe P, Ryan A. The use of irinotecan, oxaliplatin and raltitrexed for the treatment of advanced colorectal cancer: systematic review and economic evaluation. *Health technology assessment (Winchester, England)*. 2008;**12**(15):iii-ix, xi-162.

27. Poon MA, O'Connell MJ, Wieand HS, Krook JE, Gerstner JB, Tschetter LK, *et al.* Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. *Journal of Clinical Oncology*. 1991;**9**(11):1967-72.

28. Machover D, Goldschmidt E, Chollet P, Metzger G, Zittoun J, Marquet J, *et al.* Treatment of advanced colorectal and gastric adenocarcinomas with 5-fluorouracil and high-dose folinic acid. *Journal of Clinical Oncology*. 1986;**4**(5):685-96.

29. Madajewicz S, Petrelli N, Rustum YM, Campbell J, Herrera L, Mittelman A, *et al.* Phase I-II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. *Cancer Res*. 1984;**44**(10):4667-9.

30. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program Study. *Journal of Clinical Oncology*. 1989;**7**(4):425-32.

31. de Gramont A, Bosset JF, Milan C, Rougier P, Bouché O, Etienne PL, *et al.* Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. *Journal of Clinical Oncology*. 1997;**15**(2):808-15.

32. Leonard P, Seymour MT, James R, Hochhauser D, Ledermann JA. Phase II study of irinotecan with bolus and high dose infusional 5-FU and folinic acid (modified de Gramont) for first or second line treatment of advanced or metastatic colorectal cancer. *Br J Cancer*. 2002;**87**(11):1216-20.

33. Aranda E, Diaz-Rubio E, Cervantes A, Anton-Torres A, Carrato A, Massuti T, *et al.* Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with weekly high-dose 48-hour continuous-infusion fluorouracil for advanced colorectal cancer: a Spanish Cooperative

Group for Gastrointestinal Tumor Therapy (TTD) study. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 1998;**9**(7):727-31.

34. Köhne CH, Schöffski P, Wilke H, Käufer C, Andreesen R, Ohl U, *et al.* Effective biomodulation by leucovorin of high-dose infusion fluorouracil given as a weekly 24-hour infusion: results of a randomized trial in patients with advanced colorectal cancer. *Journal of Clinical Oncology*. 1998;**16**(2):418-26.

35. Levi F. Chronopharmacology of anticancer agents. In: Redfern P, Lemmer B, editors. Physiology and pharmacology of biological rhythms. Berlin: Springer; 1997. p. 299-331.

36. Oxford Cancer Intelligence Unit. Profile of Head and Neck Cancers in

England: Incidence, Mortality and Survival. London: National Cancer Intelligence Network; 2010.

37. Cancer Research UK. Stomach cancer incidence statistics. 2013 [cited 14/11/13]; Available from: www.cancerresearchuk.org/cancerinfo/cancerstats/types/stomach/incidence/.

38. Cancer Research UK. Stomach cancer survival statistics. 2012 [cited 14/11/13]; Available from: www.cancerresearchuk.org/cancer-info/cancerstats/types/stomach/survival/.

39. National Institute for Health and Care Excellence. Trastuzumab for the treatment of HER2-positive metastatic gastric cancer. TA208. 2010 [cited 22/11/2013]; Available from: http://www.nice.org.uk/TA208.

40. Cancer Research UK. Pancreatic cancer incidence statistics. 2013 [cited 14/11/13]; Available from: www.cancerresearchuk.org/cancerinfo/cancerstats/types/pancreas/incidence/.

41. Cancer Research UK. Pancreatic cancer survival statistics. 2013 [cited 14/11/13]; Available from: www.cancerresearchuk.org/cancerinfo/cancerstats/types/pancreas/survival/.

42. National Institute for Health and Care Excellence. Guidance on the use of gemcitabine for the treatment of pancreatic cancer. TA25. 2001 [cited 22/11/2013]; Available from: http://www.nice.org.uk/TA25.

43. Dissemination NCfRa. Undertaking systematic reviews of research on effectiveness: CRD guidelines for those carrying out or commissioning reviews. CRD Report 4. 1999.

44. National Institute for Health and Care Excellence. Diagnostics Assessment Programme manual. London: National Institute for Health and Care Excellence; 2011 19/11/2013]. Available from: http://www.nice.org.uk/media/A0B/97/DAPManualFINAL.pdf.

45. Merlin T, Lehman S, Hiller J, Ryan P. The "linked evidence approach" to assess medical tests: A critical analysis. *International journal of technology assessment in health care*. 2013;**29**(03):343-50.

46. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *BMJ*. 2009;**339**:b2535.

47. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, *et al.* QUADAS-2: A Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies. *Annals of Internal Medicine*. 2011;**155**(8):529-36.

48. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of epidemiology and community health*. 1998;**52**(6):377-84.

49. Husereau D, Drummond M, Petrou S, Carswell C, Moher D, Greenberg D, *et al.* Consolidated Health Economic Evaluation Reporting Standards (CHEERS) statement. *International journal of technology assessment in health care*. 2013;**29**(2):117-22.

50. DigitizeIt. 2.0 ed. Braunschweig, Germany: Bormisoft.

51. Guyot P, Ades A, Ouwens M, Welton N. Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. *BMC Medical Research Methodology*. 2012;**12**(1):9.

52. Hoyle M, Henley W. Improved curve fits to summary survival data: application to economic evaluation of health technologies. *BMC Medical Research Methodology*. 2011;**11**(1):139.

53. Tierney J, Stewart L, Ghersi D, Burdett S, Sydes M. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*. 2007;**8**(1):16.

54. DerSimonian R, Larid N. Meta-analysis in clinical trials. *Controlled Clinical Trials*. 1986;**7**:177-88.

55. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;**315**(109):629-34.

56. Harbord RM, Egger M, Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Statistics in medicine*. 2006;**25**(20):3443-57.

57. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Comparison of two methods to detect publication bias in meta-analysis. *JAMA : the journal of the American Medical Association*. 2006;**295**(6):676-80.

58. Bucher HC, Guyatt GH, Griffith LE, Walter SD. The results of direct and indirect treatment comparisons in meta-analysis of randomized controlled trials. *Journal of clinical epidemiology*. 1997;**50**(6):683-91.

59. Sutton A, Ades AE, Cooper N, Abrams K. Use of indirect and mixed treatment comparisons for technology assessment. *PharmacoEconomics*. 2008;**26**(9):753-67.

60. Mills EJ, Thorlund K, Ioannidis JP. Demystifying trial networks and network meta-analysis. *BMJ*. 2013;**346**:f2914.

61. Capitain O, Asevoaia A, Boisdron-Celle M, Poirier AL, Morel A, Gamelin E. Individual Fluorouracil Dose Adjustment in FOLFOX Based on Pharmacokinetic Follow-Up Compared With

Conventional Body-Area-Surface Dosing: A Phase II, Proof-of-Concept Study. *Clin Colorectal Cancer*. 2012;**11**(4):263-7.

62. Gamelin E, Delva R, Jacob J, Merrouche Y, Raoul JL, Pezet D, *et al*. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *Journal of Clinical Oncology*. 2008;**26**(13):2099-105.

Appendix 1. Draft search strategy

Search for clinical-effectiveness of My5-FU

Embase (Ovid), searched on 07/11/2013

1	(my5-fu* or my5fu* or "my5 fu*" or "my 5fu*" or "my 5 fu*").mp.	6
2	ondose.mp.	6
3	saladax.mp.	9
4	1 or 2 or 3	17
5	"myriad genetic*".mp.	122
6	exp immunoassay/	384759
7	(immunoassay* or (immun* adj2 assay*)).mp.	372343
8	6 or 7	467731
9	high performance liquid chromatography/	194861
10	"high performance liquid chromatography".tw.	80213
11	HPLC.tw.	128529
12	"high pressure liquid chromatography".tw.	10971
13	high speed liquid chromatography.tw.	264
14	9 or 10 or 11 or 12 or 13	254234
15	liquid chromatography/ and mass spectrometry/	21609
16	Liquid chromatography-mass spectrometry.tw.	9016
17	LC?MS*.tw.	831
18	HPLC?MS.tw.	37
19	15 or 16 or 17 or 18	26885
20	((pharmacokinetic* or PK) adj2 (dosage* or dose* or dosing or adjust* or adapt* or monitor* or select* or calculat* or guided)).mp.	29660
21	fluorouracil/pk	2850
22	fluorouracil/	104467
23	(fluorouracil* or 5-fluorouracil* or 5fluorouracil*).tw.	36712
24	(5-fu* or 5fu* or fu).tw.	31295

25	22 or 23 or 24	117313
26	exp drug dose/	407494
27	drug monitoring/ or drug clearance/	78910
28	((dose* or dosing or dosage* or fluorouracil* or 5-fluorouracil* or 5fluorouracil* or 5-fu* or 5fu*) adj2 (adjust* or adapt* or monitor* or select* or calculat* or intensi* or escalat* or modif* or reduc* or concentration* or level* or limit*)).tw.	147981
29	((drug* or blood or plasma) adj5 (monitor* or concentration* or level*) adj5 (fluorouracil* or 5-fluorouracil* or 5fluorouracil* or 5-fu* or 5fu* or fu)).tw.	652
30	("optimal drug therapy" or ("optimal drug" adj (dosage* or dose* or dosing))).tw.	330
31	26 or 27 or 28 or 29 or 30	584346
32	personalized medicine/ and exp chemotherapy/	794
33	((personal* or individual*) adj2 (chemotherap* or dosage* or dose* or dosing)).mp.	9819
34	32 or 33	10421
35	31 or 34	590807
36	5 and 25	5
37	5 and 35	5
38	36 or 37	8
39	8 and 25 and 35	242
40	((5-fu* or 5fu* or fu) adj "plasma assay*").mp.	2
41	21 and 35	1295
42	4 or 38 or 39 or 40 or 41	1536
43	14 and 25	1247
44	19 and 25	94
45	43 or 44	1314
46	35 and 45	460
47	20 and 25	316

48	42 or 46 or 47	2043

Appendix 2. Data extraction form

A. Data extraction form for primary studies in patient-based studies

Name of the reviewer:

Study details
Study ID (Ref man): First author surname: Year of publication: Country: Study design: Type of publication (e.g. full text/abstract): Study setting: Number of centres: Duration of study: Follow up period: Funding:
Aim of the study
Inclusion/exclusion criteria
Inclusion criteria: Exclusion criteria:
Participants
Total number of participants: Sample attrition/drop out: Characteristics of participants: *Mean age:*

Mean sex: *Race:* *Diagnosis:*
Method of 5-FU plasma measurement
Indication for treatment: Type of dose regimen used: Any comparison: Other interventions used:
Outcomes
Primary outcomes: Secondary outcomes: Method of assessing outcomes: Timing of assessment: Study end point: Survival analysis: Yes/No Adverse event: Yes/No Health related quality of life: Yes/No; which measures used? Length of follow up:

Number of participants	Intervention	Comparator, if present
Screened		
Randomised/Included		
Excluded		
Missing participants		

Withdrawals		
Patient's baseline characteristics	Intervention	Comparator, if present
Age		
Sex		
Weight		
BMA (range)		
Performance status		
Intermediate measures	Intervention	Comparator, if present
Accuracy of area under the curve measurements		
Proportion of patients with 5-FU plasma levels in the optimal target range		
Incidence of over and under dosing		
Frequency of dose adjustment		
Test failure rates		
Other		
Clinical outcomes	Intervention	Comparator, if present
Incidence of side effects and 5-FU toxicity: • Diarrhoea • Oral and gastrointestinal mucositis • Anaemia • Fatigue • Nausea • Vomiting • Palmar-plantar erythrodysesthesia (hand-foot syndrome)		

• Other:		
Treatment response rates		
Progression free survival • Kaplan Meier • Risk Table • Censorings		
Overall survival • Kaplan Meier • Risk Table • Censorings		
Health related quality of life		
Authors' conclusion		
Reviewer's conclusion		

B. Data extraction form for primary studies that estimate accuracy of My5-FU versus a gold standard

Name of the reviewer:

Study details
Study ID (Ref man): First author surname: Year of publication: Country: Study design: Type of publication (e.g. full text/abstract): Funding:
Aim of the study
Type and number of samples used (e.g. tumour type(s), dose regimen)
Nature of gold standard
Number and types of clinical auto-analyser(s) employed
Results
Overall findings: Bland–Altman plot (Difference plot): Correlation coefficient (equation): Influential factors: Linearity:

Precision: Accuracy: Recovery: Interference: Sample carryover: Other:
Authors' conclusion
Reviewer's conclusion

C. Data extraction form for economic studies

Name of the reviewer:

Study intervention
Objective
Design
Analytical framework (type of model):
Patient population:
Comparator:
Analytic horizon:
Perspective:
Setting:
Clinical measures:
Effectiveness measures:
Economic measures:
Methods
Health care system:
Model description:
Data sources (efficacy, resource use, costs, appropriately measured, all costs included?):
Data collection (primary data collection, if appropriate):
Probabilities:
Healthcare use:
Sensitivity analysis (allowance made for uncertainty):
Discounting (costs/benefits?):

Results
Cost of My5-FU testing:
Cost of delivering 5-FU by infusion:
Cost of side effects and 5-FU toxicity:
Treatment or hospitalisation costs:
Other:
Authors' conclusion
Reviewer's conclusion

Appendix 3. Quality assessment forms

A. Downs and Black Checklist[48]

Q1. Clear hypothesis/aim/objective clearly described

Q2. Main outcomes to be measured clearly described in the Introduction or Methods section

Q3. Characteristics of the patients included in the study clearly described

Q4. Interventions of interest clearly described

Q5. Distributions of principal confounders in each group of subjects to be compared clearly described

Q6. Main findings of the study clearly described

Q7. Estimates of the random variability in the data for the main outcomes

Q8. All important adverse events that may be a consequence of the intervention reported

Q9. Characteristics of patients lost to follow-up described

Q10. Actual probability values reported for the main outcomes except where the probability value is less than 0.001

Q11. Asked a representative sample of the population to undertake the study

Q12. Subjects who were prepared to participate who were representative of the entire population from which they were recruited

Q13. The staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive

Q14. Were identified as attempting to blind study subjects to the intervention they have received

Q15. Made an attempt to blind those measuring the main outcomes of the intervention

Q16. Made clear any results that were based on "data dredging"

Q17. Adjusted for different lengths of follow-up of patients, or in case-control studies the same time period between the intervention and outcome

Q18. Were identified as using appropriate statistical tests used to assess the main outcomes

Q19. Reliable compliance with the intervention/s

Q20. Accurate main outcome measures

Q21. Patients in different intervention groups (trials and cohort studies), or cases and controls (case-control studies), recruited from the same population

Q22. Study subjects in different intervention groups (trials and cohort studies,) or cases and controls (case-control studies), recruited over the same period of time

Q23. Study subjects randomised to intervention groups

Q24. Randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable

Q25. Adequate adjustment for confounding in the analyses from the main findings

Q26. Losses of patients to follow-up taken into account

Q27. Sufficient power was described to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%

B. Critical appraisal of the economic evaluation studies using the CHEERS checklist (adapted from Husereau et al, 2013)[49]

Title and abstract				
1 Title: Identify the study as an economic evaluation, or use more specific terms such as ``cost-effectiveness analysis``, and describe the interventions compared.				
2 Abstract: Provide a structured summary of objectives, methods including study design and inputs, results including base case and uncertainty analyses, and conclusions.				
Introduction				
3 Background & objectives: Provide an explicit statement of the broader context for the study. Present the study question and its relevance for health policy or practice decisions.				
Methods				
4 Target Population and Subgroups: Describe characteristics of the base case population and subgroups analysed including why they were chosen.				
5 Setting and Location: State relevant aspects of the system(s) in which the decision(s) need(s) to be made.				
6 Study perspective: Describe the perspective of the study and relate this to the costs being evaluated.				
7 Comparators: Describe the interventions or strategies being compared and state why they were chosen.				
8 Time Horizon: State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.				
9 Discount Rate: Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.				
10 Choice of Health Outcomes: Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.				
11a Measurement of Effectiveness - Single Study-Based Estimates: Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.				
11b Measurement of Effectiveness - Synthesis-based Estimates: Describe fully the methods used for identification of included studies and clinical effectiveness data synthesis of clinical effectiveness data.				
12 Measurement and Valuation of Preference-based Outcomes: If applicable, describe the population and methods used to elicit preferences for health outcomes.				
13a Estimating Resources and Costs - Single Study-based Economic evaluation: Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any				

adjustments made to approximate to opportunity costs.				
13b Estimating Resources and Costs - Model-based Economic Evaluation: Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.				
14 Currency, Price Date and Conversion: Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.				
15 Choice of Model: Describe and give reasons for the specific type of decision-analytic model used. Providing a figure to show model structure is strongly recommended.				
16 Assumptions: Describe all structural or other assumptions underpinning the decision-analytic model.				
17 Analytic Methods: Describe all analytic methods supporting the evaluation. This could include methods for dealing with skewed, missing or censored data, extrapolation methods, methods for pooling data, approaches to validate a model, and methods for handling population heterogeneity and uncertainty.				
Results				
18 Study parameters: Report the values, ranges, references, and if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. We strongly recommend the use of a table to show the input values.				
19. Incremental costs and outcomes: For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.				
20a Characterizing Uncertainty - Single study-based economic evaluation: Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness, parameters together with the impact of methodological assumptions.				
20b Characterizing Uncertainty - Model-based economic evaluation: Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.				
21 Characterizing Heterogeneity: If applicable, report differences in costs, outcomes or in cost-effectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects				

that are not reducible by more information.				
Discussion				
22 Study Findings, Limitations, Generalizability, and Current Knowledge: Summarize key study findings and describe how they support the conclusions reached. Discuss limitations and the generalizability of the findings and how the findings fit with current knowledge.				
Other				
23 Source of Funding: Describe how the study was funded and the role of the funder in the identification, design, conduct and reporting of the analysis. Describe other non-monetary sources of support.				
24 Conflicts of Interest: Describe any potential for conflict of interest among study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors' recommendations				

*Key: Y = yes, No = no, N/A = not applicable and * = partially completed*

C. QUADAS-2[47]

SIGN	**Methodology Checklist 5: Studies of Diagnostic Accuracy** *This checklist is based on the work of the QUADAS2 team at Bristol Univeristy (http://www.bris.ac.uk/quadas/)*		
Study identification (*Include author, title, reference, year of publication*)			
Guideline topic:		Key Question No:	
Before completing this checklist, consider: 1. Is the paper really a study of diagnostic accuracy? It should be comparing a specific diagnostic test against another, and **not** a general paper or comment on diagnosis. 2. Is the paper relevant to key question? Analyse using PICO (Patient or Population Intervention Comparison Outcome). IF NO REJECT (give reason below). IF YES complete the checklist.			
Reason for rejection: Reason for rejection: 1. Paper not relevant to key question ☐ 2. Other reason ☐ (please specify):			
Checklist completed by:			
All the questions in the following sections have associated footnotes providing short explanations behind each of the questions. Users who want more detailed explanations should consult the QUADAS-2: Background Document.			
DOMAIN 1 – PATIENT SELECTION			
Risk of bias			
In a well conducted diagnostic study…		*Is that true in this study?*	
1.1	A consecutive sequence or random selection of patients is enrolled.[i]	Yes ☐ No ☐	Can't say ☐
1.2	Case – control methods are not used.[ii]	Yes ☐ No ☐	Can't say ☐
1.3	Inappropriate exclusions are avoided.[iii]	Yes ☐ No ☐	Can't say ☐
Applicability			
1.4	The included patients and settings match the key question.[iv]	Yes ☐ No ☐	Can't say ☐
DOMAIN 2 – INDEX TEST			
Risk of bias			
In a well conducted diagnostic study…		*Is that true in this study?*	
2.1	The index test results interpreted without knowledge of the results of the reference standard.[v]	Yes ☐ No ☐	Can't say ☐
2.2	If a threshold is used, it is pre-specified.[vi]	Yes ☐ No ☐	Can't say ☐

Applicability			
2.3	The index test, its conduct, and its interpretation is similar to that used in practice with the target population of the guideline.[vii]	Yes ☐ No ☐	Can't say ☐
DOMAIN 3 – REFERENCE STANDARD			
Risk of bias			
In a well conducted diagnostic study…		*Is that true in this study?*	
3.1	The reference standard is likely to correctly identify the target condition.[viii]	Yes ☐ No ☐	Can't say ☐
3.2	Reference standard results are interpreted without knowledge of the results of the index test.[ix]	Yes ☐ No ☐	Can't say ☐
Applicability			
3.3	The target condition as defined by the reference standard matches that found in the target population of the guideline.[x]	Yes ☐ No ☐	Can't say ☐
DOMAIN 4 – FLOW AND TIMING			
Risk of bias			
In a well conducted diagnostic study…		*Is that true in this study?*	
4.1	There is an appropriate interval between the index test and reference standard.[xi]	Yes ☐ No ☐	Can't say ☐
4.2	All patients receive the same reference standard.[xii]	Yes ☐ No ☐	Can't say ☐
4.3	All patients recruited into the study are included in the analysis.[xiii]	Yes ☐ No ☐	Can't say ☐
SECTION 5: OVERALL ASSESSMENT OF THE STUDY			
5.1	**How well was the study done to minimise bias?** ***Code as follows:***[xiv]	High quality (++) ☐ Acceptable (+) ☐ Unacceptable – reject 0 ☐	
5.2	What is your assessment of the **applicability** of this study to our target population?	Directly applicable ☐ Some indirectness ☐ (Please explain in the following section for **Notes**)	
5.2	**Notes.** Summarise the authors conclusions. Add any comments on your own assessment of the study, and the extent to which it answers your question.		

[i] Studies should enrol either all eligible patients suspected of having the target condition during a specified period, or a random sample of those patients. The essential point is that investigators should have no freedom of choice as to which individual patients are or are not included.

[ii] There is evidence that studies comparing patients with known disease with a control group without the condition tend to exaggerate diagnostic accuracy.

[iii] Inappropriate exclusions may result in either overestimates (e.g., by excluding 'difficult to diagnose' patients) or underestimates (e.g., by excluding patients with 'red flags' suggesting presence of disease) of the degree of diagnostic accuracy.
[iv] Patients included in the study should match the target population of the guideline in terms of severity of the target condition, demographic features, presence of differential diagnosis or co-morbidity, setting of the study and previous testing protocols.
[v] This is similar to the question of 'blinding' in intervention studies. The index test should always been done first, or by a separate investigator with no knowledge of the outcome of the reference test.
[vi] Bias can be introduced if a threshold level is set after data has been collected. Any minimum threshold should be specified at the start of the trial.
[vii] Variations in test technology, execution, or interpretation (e.g., use of a higher ultrasound transducer frequency) may affect estimates of diagnostic accuracy.
[viii] Estimates of test accuracy are based on the assumption that the reference standard is 100% sensitive (=accurately diagnoses the target condition).
[ix] This is the similar to question 2.1, but in this case relates to making sure the reference standard is applied without any prior knowledge of the outcome of previous tests.
[x] The definition of the target condition used when testing the reference standard may differ from that used by the NHS in Scotland. E.g., threshold levels used in laboratory cultures may differ.
[xi] The index test and reference standard should be performed as close together in time as possible, otherwise changes in the patient's condition is likely to invalidate the results.
[xii] In some cases the choice of reference standard may be influenced by the outcome of the index test or the urgency of the need for diagnosis. Use of different reference standards is likely to lead to overestimates of both sensitivity and specificity.
[xiii] Not including all patients in the analysis may lead to bias as there may be some systematic difference between those lost to follow-up and those analysed.
[xiv] Rate the overall methodological quality of the study, using the following as a guide: **High quality** (++): Majority of criteria met. Little or no risk of bias. Results unlikely to be changed by further research. **Acceptable** (+): Most criteria met. Some flaws in the study with an associated risk of bias, Conclusions may change in the light of further studies. **Low quality** (0): Either most criteria not met, or significant flaws relating to key aspects of study design. Conclusions likely to change in the light of further studies.