

# Molecular testing for Lynch syndrome in people with colorectal cancer

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## Plain English summary

Lynch syndrome is an inherited condition which increases the risk of bowel cancer and other cancers. It is caused by mutations in the DNA mismatch repair genes which are responsible for correcting mistakes when DNA is copied. These mutations stop the mismatch repair system from working properly and mistakes in the DNA go uncorrected. These mistakes can eventually lead to cancer developing in the bowel and elsewhere in the body. There are no symptoms of Lynch syndrome before cancer develops. A person with Lynch syndrome has a 50:50 chance of passing it to each of their children.

If a person is known to have Lynch syndrome then they can be offered surveillance and/or other risk-reducing measures which can reduce their risk of developing cancer, or pick up cancers at an early stage when they are more treatable. Their close relatives can also be tested to see if they have Lynch syndrome.

It is possible to test any person's DNA from their blood to see whether they have Lynch syndrome, but these tests are expensive and can produce results which are difficult to interpret.

Other tests are available which use cancer tissue and which give an indication of whether a person might have Lynch syndrome. The two main tests of this kind are microsatellite instability (MSI) testing and immunohistochemistry (IHC). Clinical studies to find out how accurate these tests are have mainly been done in bowel cancer patients. These tests can be less expensive but are not accurate enough to diagnose Lynch syndrome properly. If one or more of these tests suggest a person has Lynch syndrome then further testing is used for proper diagnosis. IHC can also help to direct further testing and interpret the results.

It has been suggested that performing MSI or IHC testing on all new bowel cancers, and then offering genetic testing for Lynch syndrome to some patients, could result in better diagnosis of Lynch syndrome, reduce the risk of cancer, prevent early deaths and be a good use of NHS resources.

This research will look at the evidence for how accurate tests for Lynch syndrome are, and how effective and cost-effective these tests would be if used in the NHS.

# 1 Background

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## 1.1 Lynch syndrome

### 1.1.1 History

The earliest recorded clinical identification of Lynch syndrome was by Aldred Warthin, who studied a cancer family (subsequently named “G”) from 1895 and published in 1913.<sup>1</sup> The specific phenotype, termed hereditary non-polyposis colorectal cancer (HNPCC), was described in the mid-1960s by Henry Lynch, and consisted of an autosomal dominant inheritance pattern, with high but incomplete penetrance of colorectal cancer (at an earlier age than in the general population), more right-sided colorectal cancers (further from the rectum) and multiple cancers. Cancers outside the colorectum (extracolonic cancers) were also identified as being at higher risk in these families.<sup>2</sup> Families with such cancers have historically been termed as Lynch II or HNPCC II families (with Lynch/HNPCC I referring to families with few extracolonic cancers).<sup>3</sup>

In 1991, the Amsterdam criteria were devised<sup>4</sup> (and subsequently revised in 1999,<sup>5</sup> primarily to include extracolonic cancers) in order to provide uniformity of recruitment in collaborative studies of HNPCC.

In 1993, the molecular basis for Lynch syndrome began to be understood, with the identification of cancer susceptibility loci on 2p and 3p through linkage analyses, the description of the microsatellite instability (MSI) phenotype, and the discovery of pathogenic mutations in *MSH2*. Pathogenic mutations in *MLH1* and *PMS2* were discovered in 1994, followed by *MSH6* in 1997.<sup>6</sup>

In 1996, the Bethesda guidelines<sup>7</sup> (and revised Bethesda guidelines<sup>8</sup>) were proposed as a means of identifying patients suitable for MSI testing and subsequent mutation screening.

In 2009, certain constitutional mutations in the *EPCAM* gene (located very close to *MSH2* on chromosome 2) were shown to result in epigenetic silencing of *MSH2* through promoter hypermethylation.<sup>9</sup>

Readers with further interest in the history of Lynch syndrome are referred to Henry T Lynch and Jane F Lynch (2004)<sup>6</sup> for a comprehensively referenced history of Lynch syndrome to 2003.

### 1.1.2 Aetiology

Lynch syndrome is caused by constitutional pathogenic mutations in the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*) or by certain mutations in the *EPCAM* gene. An individual with Lynch syndrome still has a functioning MMR system generally since they inherit a functioning allele from one parent in addition to the non-functioning allele from the parent with Lynch syndrome. There is, however, a loss of resilience, and somatic loss of function in the other allele leads to MMR deficiency, which leads to tumorigenesis through the microsatellite instability pathway, in which replication errors proliferate.

Mutations in the different genes can lead to different cancer risks (generally mutations in *MLH1* and *MSH2* carry the highest cancer risk).<sup>10</sup>

### 1.1.3 Epidemiology

The most recent estimates suggest that the prevalence of Lynch syndrome MMR mutations is 1 in 370, and responsible for around 2.8% of colorectal cancer.<sup>11</sup> On this basis, an estimated 175,000 people in the UK has Lynch syndrome, and this leads to over 1,100 colorectal cancers per year across the UK.

### 1.1.4 Management

The risks of colorectal and endometrial cancer are high in individuals with Lynch syndrome, with estimates of 25–70% for the lifetime risk, in addition to increased risk of other cancers, such as ovarian and gastric cancer.<sup>12</sup>

Many organisations have made recommendations as to the appropriate management of individuals with Lynch syndrome. The Mallorca group of European experts recently made the following recommendations:

- Colorectal surveillance (regular colonoscopy) has been shown to be effective with a 3-year interval, an interval of 1–2 years is recommended from age 20–25 years;
- Gynaecological surveillance has not been shown to be effective, but should be offered to mutation carriers from age 35–40 years with a discussion of the potential risks and benefits;
- Prophylactic hysterectomy and bilateral salpingo-oophorectomy (H-BSO) should be offered to mutation carriers after they have completed their families (especially after the age of 40 years) with a discussion of the potential risks and benefits;
- Prophylactic H-BSO should also be discussed if surgery for colorectal cancer is scheduled;
- Surveillance for other extracolonic cancers should only be performed in a research setting;
- More extensive surgery for colorectal cancer in mutation carriers should be discussed, given the substantial risk of a second colorectal cancer, with a discussion of the potential risks and benefits;
- Individuals with Lynch syndrome should be advised to maintain a normal weight and refrain from smoking;
- Regular aspirin is effective in reducing the incidence of cancer in individuals with Lynch syndrome, and low-dose daily aspirin should be offered with a discussion of the potential risks and benefits;
- Cancer geneticists and genetic counsellors should be prepared to discuss prenatal and preimplantation genetic diagnosis of Lynch syndrome during genetic counselling;
- Professionals should be aware of the risk of psychosocial problems before and after genetic testing and during follow-up and surveillance visits.<sup>12</sup>

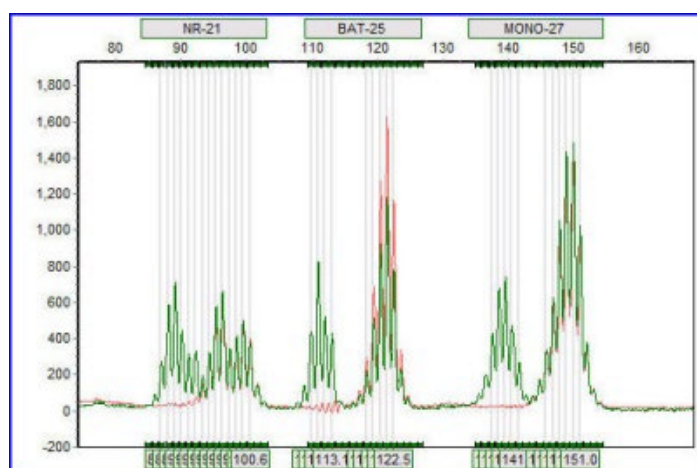
## 1.2 Diagnostic tests for Lynch syndrome

### 1.2.1 Tumour-based tests

#### **Microsatellite instability (MSI) testing**

DNA microsatellites are short repetitive sequences found throughout the human genome. When the DNA mismatch repair system is defective the length of these sequences can become variable as errors go uncorrected; this is known as microsatellite instability.

Molecular MSI testing involves polymerase chain reaction (PCR) amplification of DNA markers from a tumour tissue sample and a healthy tissue sample (see *Figure 1*). The tissue samples must be microdissected before DNA is extracted and amplified.



**Figure 1: Example of molecular microsatellite instability testing. Red trace shows normal tissue, green trace tumour tissue.** Source: Microsatellite Instability in GeneMarker [[https://commons.wikimedia.org/wiki/File:Microsatellite\\_Instability\\_in\\_GeneMarker.jpg](https://commons.wikimedia.org/wiki/File:Microsatellite_Instability_in_GeneMarker.jpg)]

Mono- and dinucleotide markers are the most frequently used. Microsatellite instability is characterised as MSI-High ( $\geq 30\%$ ), MSI-Low ( $< 30\%$  but  $> 0\%$ ) or MS-Stable ( $0\%$ ) according to the proportion of markers indicating MSI, although there is ongoing debate as to whether MSI-Low should be considered phenotypically as indicating microsatellite instability. The National Cancer Institute (NCI) recommended a panel of five markers, known as the Bethesda/NCI markers,<sup>8</sup> but other markers are in use and individual laboratories may develop their own panels (see *Table 1*).

**Table 1: Microsatellite markers used in molecular microsatellite instability testing**

Panel	Mononucleotide	Dinucleotide	Other	Notes
Bethesda/NCI <sup>8</sup>	BAT-25, BAT-26	D2S123, D5S346, D17S250		If only dinucleotide repeats are mutated, test a secondary panel of microsatellite markers with mononucleotide markers to exclude MSI-Low
NCI suggested markers for	BAT-40, MYCL			

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secondary panel<sup>8</sup>

Promega MSI  
Analysis System  
v1.2<sup>13</sup>

BAT-25, BAT-26,  
MONO-27, NR-  
21, NR-24

Penta C, Penta D

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MSI-High is associated with Lynch syndrome, but is also present in around 15% of sporadic cancers.<sup>14</sup>

### **MMR immunohistochemistry**

MMR immunohistochemistry (IHC) tests for the presence or absence of MMR proteins in colorectal cancer tissue. Antibodies for the MMR proteins (and potentially for EpCAM) are used to stain tumour tissue and healthy tissue (as a control). If nuclear staining is present for all MMR proteins, this suggests the MMR system is intact, whereas abnormal nuclear staining for one or more MMR proteins suggests MMR deficiency. The pattern of staining across the MMR proteins also indicates the MMR genes which may have pathogenic mutations (see Table 2, page 11). Abnormal nuclear staining can take the form of total absence or weak/patchy staining.<sup>15</sup>

Some pathogenic mutations may result in loss of MMR function in the protein but still allow its expression as a stable protein and binding to the relevant antibody, resulting in a “false negative” result.

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

Microsatellite instability and abnormal MLH1 immunostaining can be caused in sporadic (i.e., not due to Lynch syndrome) colorectal cancer by somatic hypermethylation of the *MLH1* promoter,<sup>15</sup> and this is also associated with the tumour marker *BRAF* V600E (a specific mutation in the *BRAF* gene). *BRAF* V600E is very rarely found in colorectal cancers due to Lynch syndrome, but around half of MSI-High sporadic colorectal cancers do not have *BRAF* V600E. *MLH1* hypermethylation is observed in many MSI-High sporadic colorectal cancers but is also observed in some colorectal cancers due to Lynch syndrome.<sup>15</sup>

*BRAF* V600E and *MLH1* methylation testing may be used after MSI testing or IHC to attempt to exclude “false positive” results without excluding many “true positive” results. This should therefore reduce the number of patients receiving genetic counselling and testing (which has cost implications and can lead to psychosocial problems).

### **1.2.2 Constitutional DNA tests**

The gold standard for diagnosis of Lynch syndrome is comprehensive screening for constitutional mutations in the MMR genes and *EPCAM*. This screening is conducted using a DNA sequencing method to detect point mutations and small insertions and deletions and multiplex ligation-dependent probe amplification (MLPA) to detect large structural DNA abnormalities, such as genomic deletions, duplications and rearrangements.<sup>16</sup>

Comprehensive screening for constitutional mutations should accurately detect all known Lynch syndrome-causing mutations. In some cases a novel mutation may be identified of uncertain significance; in this case there are recent guidelines which should guide classification.<sup>17</sup> Nevertheless, some existing variants are of unknown clinical significance, and identification of these in an individual with colorectal cancer may lead to uncertainty in the interpretation of results and subsequent clinical management.

### 1.2.3 Clinical criteria

Note: These should not be considered as diagnostic tests for Lynch syndrome, but are presented here for reference as they are mentioned in several guidelines.

The Amsterdam and Amsterdam II criteria were designed to identify families with Lynch syndrome for research studies, while the Bethesda and revised Bethesda guidelines are designed to identify individuals suitable for evaluation for MMR deficiency through tumour testing.

*Table 5* and *Table 6* in Appendix 1 give further details of these clinical criteria.

## 1.3 Recent health technology assessments and guidelines on testing for Lynch syndrome

### 1.3.1 PenTAG systematic review and economic evaluation, published 2014

In 2012–13 the Peninsula Technology Assessment Group (PenTAG) conducted a systematic review and economic evaluation of diagnostic strategies for Lynch syndrome in early-onset (aged under 50 years) colorectal cancer patients for the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme.<sup>16</sup>

Ranges of sensitivity and specificity estimates were produced for MSI and IHC, but it was noted that most of the included studies were at risk of bias because the reference standard was not conducted on all participants.

The PenTAG economic evaluation consisted of a decision analytic model in which early-onset colorectal cancer patients were either not tested for Lynch syndrome, or were offered a testing strategy comprising constitutional MMR mutation testing with or without tumour-based testing (for population enrichment or triage). Relatives of patients diagnosed with Lynch syndrome were offered testing for the family mutation. All individuals diagnosed with Lynch syndrome were offered colonoscopic surveillance, as well as prophylactic gynaecological surgery (women only). A testing strategy with MSI and *BRAF* V600E mutation testing as tumour-based tests was predicted to be cost-effective with a cost-effectiveness threshold of £20,000 per QALY.

The existing PenTAG report does not wholly address the decision problem considered here (see *Section 2, Decision problem*) because the population considered was early-onset colorectal cancer patients rather than all colorectal cancer patients. The prevalence of Lynch syndrome in all colorectal cancer patients is significantly lower than the prevalence in early-onset colorectal cancer patients,<sup>18</sup> and the prevalence of Lynch syndrome in the input population was identified as being an important driver of cost-effectiveness.

### 1.3.2 US Agency for Healthcare Research and Quality systematic review, published 2007

A systematic review was prepared by the Tufts-New England Medical Center Evidence-based Practice Center for the US Agency for Healthcare Research and Quality (AHRQ).<sup>3</sup> The overarching key question for the review was whether screening for HNPCC in newly-diagnosed colorectal cancer patients leads to improved outcomes for them or their family members, and whether it is useful in medical, personal or public health decision making.

The overarching key question was decomposed into ten more specific questions, of which some concerned the diagnostic performance of MSI and IHC. MSI was associated with moderate to good diagnostic performance: after exclusion of studies with fewer than 40

patients in the 2 × 2 table, the summary estimate of sensitivity was 0.83 (95% CI, 0.65–0.92) and of specificity was 0.87 (95% CI, 0.80–0.91). Summary sensitivity and specificity were also estimated for IHC, based on “good or fair quality studies”, with sensitivity 0.74 (95% CI, 0.54–0.87) and specificity 0.77 (95% CI, 0.61–0.88).

### **1.3.3 Evaluation of Genomic Applications in Practice and Prevention (EGAPP) evidence review and recommendations, published 2009**

The EGAPP Working Group initiated a supplementary evidence review<sup>19</sup> to be considered alongside the review commissioned through AHRQ<sup>3</sup> and simple economic modelling, to develop recommendations regarding the use of testing strategies to identify Lynch syndrome.<sup>20</sup>

MSI was estimated to have sensitivity 0.85 (95% CI, 0.75–0.92) to detect Lynch syndrome caused by *MLH1* mutations, 0.85 (95% CI, 0.73–0.93) to detect Lynch syndrome caused by *MSH2* mutations, and 0.69 (95% CI, 0.46–0.85) to detect Lynch syndrome caused by *MSH6* mutations. The specificity of MSI was estimated to be 0.902 (95% CI, 0.870–0.927).<sup>19</sup>

The EGAPP Working Group found sufficient evidence to recommend offering genetic testing for Lynch syndrome to individuals with newly-diagnosed colorectal cancer, but could not recommend a specific testing strategy.<sup>20</sup>

### **1.3.4 The Mallorca group literature review and guidelines, published 2013**

The Mallorca group reviewed a number of studies which evaluated prospective screening of colorectal or endometrial cancer for Lynch syndrome, and recommended testing all individuals with colorectal/endometrial cancer (or all individuals diagnosed with colorectal/endometrial cancer under the age of 70 years) with MSI or MMR IHC.<sup>12</sup>

### **1.3.5 US Multi-Society Task Force on Colorectal Cancer literature review and guidelines, published 2014**

The US Multi-society task force on colorectal cancer performed a literature review and developed guidelines relating to the identification and management of Lynch syndrome. They recommended that testing of newly-diagnosed colorectal cancer for MMR proficiency should be performed using four-panel IHC (followed by *BRAF* testing or *MLH1* methylation testing if loss of *MLH1* is shown) or MSI. The guideline suggests testing all colorectal cancer patients or those aged under 70 years. The task force recommended genetic evaluation for Lynch syndrome in: individuals with a MMR-deficient tumour (without evidence of *MLH1* promoter methylation); endometrial cancer diagnosed under age 50; relatives for a known family MMR mutation; individuals fulfilling the Amsterdam or revised Bethesda criteria; individuals with a personal risk  $\geq 5\%$  according to risk prediction models.<sup>21</sup>

### **1.3.6 Recent American and European guidelines**

The US National Comprehensive Cancer Network regularly produces an issue of Clinical Practice Guidelines in Oncology on “Genetic/familial high-risk assessment: colorectal [cancer]”, with its latest edition in July 2015.<sup>22</sup> The guidelines recommend testing any individual fulfilling at least one of the following criteria: meeting the Bethesda criteria or from a family meeting the Amsterdam criteria; endometrial cancer aged under 50 years; known Lynch syndrome in the family;  $\geq 5\%$  risk of Lynch syndrome based on risk prediction models. If tumour tissue is available then testing should initially be done using IHC or MSI. *BRAF* or *MLH1* methylation studies are recommended for individuals with *MLH1* abnormalities on IHC.

The European Society for Medical Oncology Clinical Practice Guidelines for familial risk colorectal cancer recommend that tumour MMR testing (by MSI or IHC) should be conducted for all individuals diagnosed with colorectal cancer under age 70 years, and all individuals aged over 70 years meeting the revised Bethesda guidelines. They also recommend *BRAF* V600E or *MLH1* methylation testing for loss of MLH1 on IHC.<sup>23</sup>

The Royal College of Pathology dataset for colorectal cancer histopathology reports indicates that MMR IHC is a core dataset item for patients aged under 50 years at the time of diagnosis, and for patients with adenocarcinomas with poorly-differentiated morphology or other morphological features of MMR deficiency.<sup>24</sup>

## 2 Decision problem

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### 2.1 Purpose of the decision to be made

To evaluate the effectiveness and cost-effectiveness of polymerase chain reaction (PCR) based assessment of microsatellite instability (MSI) or immunohistochemical assessment of mismatch repair (MMR) proficiency to indicate the presence of Lynch syndrome in individuals newly diagnosed with colorectal cancer.

### 2.2 Clear definition of the interventions

MSI testing: PCR-based MSI testing as carried out by CPA-accredited regional genetics laboratories using validated in-house tests or the Promega MSI Analysis System (a commercial test).

IHC testing: Immunohistochemical testing for MMR proficiency using antibodies for MMR proteins.

### 2.3 Populations and relevant subgroups

The intervention will be applied to individuals newly diagnosed with colorectal cancer. If evidence permits, age-defined colorectal cancer patient sub-populations will be included as follows: > 70 years old, < 70 years old, < 60 years old, < 50 years old (as specified in the NICE scope).

Diagnosis of Lynch syndrome in an individual is usually followed by attempts to diagnose Lynch syndrome in their relatives through cascade testing, and as such diagnosis of Lynch syndrome also has consequences for health spending and health outcomes of relatives, and these will be included within the decision problem although the intervention is only applied to individuals newly diagnosed with colorectal cancer.

Colorectal cancer is rarely diagnosed in children and adolescents, even with Lynch syndrome,<sup>10</sup> and most guidelines suggest that risk-reducing measures should not be initiated until age 20–25 years.<sup>12</sup> For these reasons, it will be assumed that only adult relatives will be offered testing for Lynch syndrome in addressing the decision problem, although colorectal cancer patients aged <18 years may be offered testing in line with guidance or clinical expert opinion.

### 2.4 Place of the intervention in the treatment pathway(s)

MSI and IHC testing are conducted on tumour tissue. This is usually obtained from tumour tissue removed during surgical treatment, but can sometimes be retrieved through preoperative biopsy. A histopathologist selects tissue for testing (tumour tissue and normal tissue) and performs microdissection for MSI or sectioning and staining for IHC. Automatic immunostaining is recommended for IHC to reduce variation. Microdissected samples for MSI testing are then processed by a laboratory genetics centre who perform PCR-based MSI testing (and any other indicated molecular genetics tests) and report to the histopathologist. The histopathologist reports findings to the cancer team (usually a consultant colorectal surgeon) along with any recommendations for further testing.

If the results of MSI and/or IHC testing are suggestive of Lynch syndrome there may be further tumour tissue based tests ordered (e.g., immunohistochemistry, *BRAF* V600E

mutation testing, *MLH1* methylation testing), or the patient may be referred directly to clinical genetics.

Clinical genetics will discuss the findings with the patient, describe Lynch syndrome and take a detailed family history (pre-test genetic counselling). If the genetics team and the patient agree that constitutional MMR mutation testing is appropriate then a blood sample will be sent to laboratory genetics for appropriate testing.

If a pathogenic constitutional MMR mutation consistent with existing findings is found then Lynch syndrome is diagnosed and the genetics team communicate this to the cancer team to adapt the follow-up and surveillance plans for the patient. The genetics team will investigate the possibility of identifying the same mutation in close relatives of the patient (known as predictive testing).

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

## **2.5 Relevant comparators**

Interventions will be compared against each other and against two further comparator “strategies”.

The first strategy is not to attempt to identify Lynch syndrome. While this will not be an effective approach to identifying Lynch syndrome, it may yet be a cost-effective use of NHS resources.

The second strategy is to offer direct constitutional MMR mutation testing to all newly-diagnosed colorectal cancer patients.

Family history tests, such as the Amsterdam and Bethesda criteria, and more advanced risk models (MMRpro, MMRpredict and PREMM<sub>1,2,6</sub>) will not be considered as preliminary tests for Lynch syndrome, due to concerns regarding the accuracy, reliability and completeness of the information gathered in the likely setting of secondary care.<sup>19</sup>

## **2.6 Key factors to be addressed**

### **2.6.1 Clinical outcomes**

*Key Question:* How effective are MSI and IHC (followed by subsequent testing as clinically indicated) for identifying Lynch syndrome through universal testing of newly-diagnosed colorectal cancers (clinical validity), and how does universal testing affect key clinical outcomes, including the incidence of Lynch syndrome-related cancers, health-related quality of life, and overall life expectancy (clinical utility)?

### **2.6.2 Cost outcomes**

*Key Question:* Is universal testing of newly-diagnosed colorectal cancers using MSI and/or IHC (and subsequent testing as clinically indicated) to identify Lynch syndrome an effective use of limited NHS resources?

### 2.6.3 Key challenges

#### **Variants of uncertain significance**

Constitutional MMR mutation screening does not always give conclusive results. One of the possible results which leads to uncertainty is the discovery of a variant of uncertain significance (VUS). Such a variant cannot be demonstrated to be pathological or non-pathological, and therefore it is not possible to make a diagnosis and immediate recommendations for management, such as colorectal surveillance. When a VUS is discovered, this is usually pursued by testing for the variant in other family members with Lynch syndrome-related cancers (or by testing stored tumour tissue for MMR deficiency). The discovery of a VUS could have a significant psychosocial impact on the patient and their family, and can also lead to surveillance of an inappropriate level (e.g., intensive surveillance for a variant which is in fact non-pathogenic or reduced surveillance for a variant which is in fact pathogenic). The discovery of a VUS will also have significant cost implications, as further genetic counselling and laboratory testing are needed.

#### **Microsatellite instability testing**

There is known to be heterogeneity in the composition of microsatellite markers in MSI panels (both in the nature and number of markers), which may lead to differences in test performance and/or threshold effects. There may also be differences in how additional markers are tested on the basis of results from the primary panel.

It also appears that MSI-Low, while uncommon in Lynch syndrome caused by *MLH1* or *MSH2* mutations, is more common in Lynch syndrome caused by *MSH6* (and presumably *PMS2*) mutations. Some studies may not report MSI-Low separately (bundling it with MSS or MSI-High) which may lead to difficulties in comparing across studies.

#### **Immunohistochemistry**

Immunohistochemistry panels may use two MMR antibodies (usually *MLH1* and *MSH2*) or four antibodies (*MLH1*, *MSH2*, *MSH6* and *PMS2*). A panel with only *MLH1* and *MSH2* antibodies is unlikely to detect MMR deficiency in *MSH6* and *PMS2* and will be expected to have lower sensitivity than a four-antibody panel.

It is also possible to consider multiple measures of the performance of an IHC panel. The expected relationship between MMR gene mutation and IHC staining is given in Table 2. If, for example, IHC staining is absent for *MLH1* but then a constitutional mutation is found in *MSH2*, should this be considered a true positive?

**Table 2: Expected IHC staining pattern according to MMR gene mutated**

MMR gene mutated	MLH1 stain	MSH2 stain	MSH6 stain	PMS2 stain	EPCAM stain
<i>MLH1</i>	–	+	+	–	+
<i>MSH2</i>	+	–	–	+	+
<i>MSH6</i>	+	+	–	+	+
<i>PMS2</i>	+	+	+	–	+
<i>EPCAM</i>	+	–	+	+	+/-

**Key:** +, (usually) present; –, (usually) absent

**Sources:** Shia 2008,<sup>25</sup> Kloor et al. 2011<sup>26</sup> and Tuttlewska et al. 2013<sup>27</sup>

In addition, patchy or weak patterns of staining can also be indicative of MMR deficiency,<sup>15</sup> but it is possible that these patterns may be interpreted differently across studies.

Finally, test failures are an issue with immunohistochemistry. Palomaki et al. counted six test failures across a number of studies (total 136 patients), corresponding to a failure rate of 4.4%.<sup>19</sup> In some cases it may be possible to obtain additional tumour tissue (from the same cancer, or a different cancer in the same individual, or a cancer in a close family member) for repeat testing.

## **2.7 Areas of agreement at the scoping workshop that are outside the scope of the appraisal and therefore do not require any detailed assessment (e.g. key factors for which evidence is already accepted).**

The External Assessment Group (EAG) will not consider whether diagnostic testing for Lynch syndrome in a newly-diagnosed colorectal cancer patient can be used to direct primary surgery (e.g., to include a risk-reducing element such as more extensive colorectal surgery or simultaneous hysterectomy and bilateral salpingo-oophorectomy), since it is not believed that a diagnosis could be achieved in a sufficiently timely manner.

The EAG will not consider the management of patients with a family history indicating Lynch syndrome (e.g., meeting Amsterdam II criteria), but with no causative MMR gene mutation found after comprehensive genetic testing (includes the group termed “Familial colorectal cancer Type X”).

### 3 Report methods for assessing the outcomes arising from the use of the interventions

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A systematic review of the diagnostic test accuracy of MSI and IHC (each with or without *BRAF* V600E mutation testing and with or without *MLH1* methylation testing) will be conducted. A supplementary structured review of end-to-end studies of universal testing with MSI and/or IHC will also be conducted.

In addition to these reviews, the health economic model (*Section 4, page 18*) will be used to extrapolate outcomes.

#### 3.1 Population

For both the review of diagnostic test accuracy and the review of end-to-end studies, the population will be colorectal cancer patients. These may be newly diagnosed or retained samples, unless there are concerns that storage methods would adversely affect test accuracy.

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

Studies in which clinical or family history criteria are used to select colorectal cancer patients will be eligible for inclusion under certain circumstances (see *Section 3.5*). Depending on the availability of data these may be considered in subgroup analyses.

#### 3.2 Interventions and comparators (index tests)

The interventions to be considered are:

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

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

For the review of diagnostic test accuracy, studies will be eligible for inclusion if one or more intervention is assessed versus a reference standard. For the review of end-to-end studies, studies will be eligible for inclusion if an intervention is compared with a comparator strategy (see *Section 2.5, p. 10*) and the reference standard is used as part of the diagnostic strategy in both groups/study arms.

#### 3.3 Reference standard

The reference standard is constitutional MMR mutation testing. This will include DNA sequencing as a minimum. MLPA will be required (studies will also be eligible for inclusion if MLPA is only conducted when sequencing finds no clearly pathogenic mutations). For older studies, another appropriate technique for detecting large genomic abnormalities should be included. Studies in which IHC results direct the MMR genes to be tested will be eligible for inclusion (e.g., if *MLH1* is not tested when only MSH2 and MSH6 proteins are absent on

IHC). Studies in which only founder mutations are sought will not be eligible for inclusion since these are not prevalent in the UK.

Unless the aim of a study is to investigate the test accuracy of an index test in individuals with mutations in a particular MMR gene, studies will need to test *MLH1*, *MSH2* and *MSH6* as a minimum (unless IHC results direct otherwise).

### 3.4 Outcomes

For the review of diagnostic test accuracy, the outcomes to be assessed for interventions and comparators are:

- Sensitivity
- Specificity
- Likelihood ratio for positive test result (LR<sup>+</sup>)
- Likelihood ratio for negative test result (LR<sup>-</sup>)
- Positive predictive value (PPV)
- Negative predictive value (NPV)
- Diagnostic yield (also known as test positivity rate or apparent prevalence)
- Test failure (non-informative test result) rate.

For the review of end-to-end studies the outcomes to be assessed may include:

- Number of individuals receiving MSI and/or IHC testing
- Number of individuals receiving subsequent tumour-based tests
- Number of individuals receiving constitutional MMR mutation testing
- Number of cascade tests on relatives
- Number of Lynch Syndrome diagnoses
- Number of colonoscopies
- Morbidity, mortality and/or life expectancy
- Costs associated with interventions and comparators
- Health-related quality of life.

### 3.5 Study design

The ideal study design for evaluating the test accuracy of MSI and IHC for Lynch syndrome in colorectal cancer patients would be to recruit newly-diagnosed colorectal cancer patients consecutively (or a random sample), perform the index test(s) and reference standard on all participants and construct 2 × 2 tables for each index test, and if more than one index test is evaluated, to give appropriate cross-tabulation.

Single-gate diagnostic studies such as these will be eligible for inclusion.

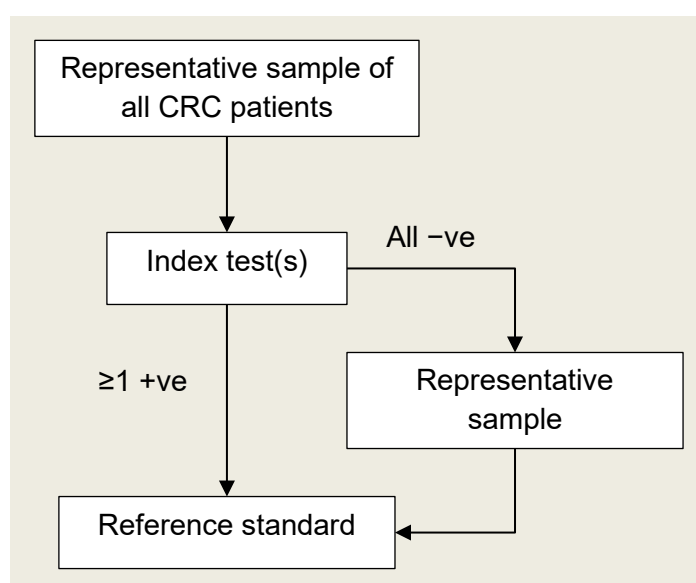
It is known, however, that many studies of test accuracy for MSI and IHC do not perform the reference standard on all participants due to the expense of testing.<sup>19</sup> Some studies instead recruit only from high-risk populations (e.g., from clinical genetics clinics, or those meeting Amsterdam or Amsterdam II criteria), or only apply the reference standard when one or more

index test is positive. Some studies only recruited colorectal cancer patients diagnosed below a certain age (since Lynch syndrome has higher prevalence in early-onset colorectal cancer than in general colorectal cancer).

When studies recruit only from high-risk populations this obviously would lead to biased estimates of PPV, NPV and yield. It will also possibly lead to biased estimates of sensitivity and specificity due to spectrum bias,<sup>28</sup> but in a previous review this did not appear to lead to significant bias in estimates of sensitivity.<sup>19</sup>

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

Studies which recruit a representative sample of all colorectal cancer patients, but do not apply the reference standard to all patients, will be included if the reference standard is applied to all patients testing positive for one or more index test and to a representative (e.g., random) sample of patients testing negative for all index tests (see *Figure 2*).



**Figure 2: Example eligible study design**

Two-gate diagnostic studies will be excluded from the review, because these designs are subject to spectrum bias.

Other study designs will only be eligible for inclusion in the review of diagnostic test accuracy if they can provide an estimate of sensitivity or specificity for one or more index test that is at minimal risk of bias.

For the review of end-to-end studies, randomised or non-randomised, controlled clinical trials will be included.

Non-experimental studies, preclinical studies, animal studies and studies published *only* in abstract form will not be eligible for inclusion (abstracts associated with full papers which are eligible for inclusion will be examined for additional information from the study) in either review. Systematic reviews will not be included themselves, but their bibliographies will be examined for potentially includable studies.

### **3.6 Search strategy**

The search strategy for both reviews will include the following sources:

- Searching of electronic databases:
  - MEDLINE (Ovid)
  - MEDLINE In-Process & Other Non-Indexed Citations (Ovid)
  - Embase (Ovid)
  - Web of Science (Thomson Reuters)
  - CENTRAL (The Cochrane Library)
  - Cochrane Database of Systematic Reviews (The Cochrane Library)
  - HTA Database (The Cochrane Library)
  - Health Management Information Consortium (Ovid)
- The review by Bonis et al.<sup>3</sup>
- [For the review of diagnostic test accuracy only] The reviews by Bonis et al.,<sup>3</sup> Palomaki et al.,<sup>19</sup> Snowsill et al.<sup>16</sup> and Vasen et al.<sup>12</sup> and any other systematic reviews identified
- Backward and forward citation chasing on included studies

Database searches for both reviews will comprise population terms for Lynch syndrome or hereditary non-polyposis colorectal cancer (adapted from the previous PenTAG search terms<sup>16</sup>), and intervention terms for MSI or IHC. A sample search strategy is given in Appendix 2.

For the test accuracy searches, methodological filters will not be used to limit study designs retrieved, since these have been shown to reduce sensitivity.<sup>29</sup> For the end-to-end study searches, methodological filters will be used to limit study design to controlled clinical trials and randomised clinical trials.

Searches will also be limited to human-only populations and English language publications.

Searches will be date-limited to 2006 onwards, since studies published pre-2006 are very likely to have been included by the reviews by Bonis et al.<sup>3</sup> (test accuracy and end-to-end studies) and Palomaki et al.<sup>19</sup> (test accuracy studies) which both searched up to 2007. Publication pre-2006 will not be an exclusion criteria.

Computer assisted deduplication will be performed.

Studies will initially be screened according to title and abstract for potential inclusion. For the systematic review of diagnostic test accuracy, the screening will be done independently by at least two experienced systematic reviewers. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary.

Full texts will be sought for any studies not excluded by title and abstract screening. These full texts will be screened independently by at least two experienced systematic reviewers for ultimate inclusion in the review. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary.

For the supplementary structured review of end-to-end studies, screening of titles and abstracts, and full text screening, will be done by one experienced reviewer.

### **3.7 Data extraction strategy**

For the systematic review of test accuracy, data will be extracted by one experienced systematic reviewer and checked by a second experienced systematic reviewer. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary. For the review of end-to-end studies, data will be extracted by one experienced systematic reviewer.

Standardised data extraction tables will be developed for both reviews.

### **3.8 Quality assessment strategy**

Diagnostic test accuracy studies will be quality assessed using the QUADAS-2 tool.<sup>30</sup> Assessments will be conducted by one experienced systematic reviewer and checked by another, with disagreements resolved by discussion, with the involvement of a third reviewer if necessary. End to end studies will be assessed for risk of bias by one reviewer, based upon CRD guidance.<sup>31</sup>

### **3.9 Methods of analysis/synthesis**

Where outcomes of interest are not directly reported in studies but can be reliably imputed from information provided, imputed values will be calculated and presented.

Results across studies will be tabulated. If any studies are known to have overlapping participants (e.g., because they recruit from the same registry) this will be highlighted and accounted for where possible in any data synthesis.

For the review of test accuracy, studies which provide estimates of both sensitivity and specificity will have their point estimates plotted in ROC space. If appropriate, these estimates may be synthesised following methodology described in the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy.<sup>32</sup>

## 4 Report methods for synthesising evidence of cost-effectiveness

The previous technology appraisal by PenTAG of strategies to diagnose Lynch syndrome<sup>16</sup> included a systematic review of economic evaluations and the development of an economic model. The systematic review of economic evaluations was broad and capable of identifying studies answering the present decision problem. The economic model included a narrower patient population, since it only considered diagnosing Lynch syndrome in early-onset (aged <50 years) colorectal cancer patients.

The systematic review of economic evaluations will be updated to incorporate any new evidence published since the review was conducted.

The economic model will be modified to include all newly-diagnosed colorectal cancer patients and to ensure that all diagnostic strategies considered are in line with the decision problem and with current or potential clinical practice. The diagnostic performance of the tests will also be re-estimated based on the review of test accuracy and the costs of tests, procedures, treatments and other costs will be updated.

### 4.1 Identifying and systematically reviewing published cost-effectiveness studies

An update to the previous systematic review of economic evaluations by PenTAG<sup>16</sup> will be conducted. Since it is known that there are cost-effectiveness studies reporting results where health effects are measured in (discounted) life years or quality-adjusted life years (QALYs) there is little value in including studies with health effects measured in natural units (e.g., MMR mutations detected) and therefore only economic evaluations in which health effects are measured in life years or QALYs will be sought and included.

#### 4.1.1 Population, intervention, comparators, outcomes and study designs

Table 3 shows the inclusion criteria for the systematic review of published cost-effectiveness studies, compared to the inclusion criteria for the previous PenTAG review. The inclusion criteria for the current review are narrowed to address the decision problem, meaning that searches only need to be run for dates after the previous PenTAG review.

**Table 3: Inclusion criteria for the systematic review of published cost-effectiveness studies**

PICOS criteria	Previous PenTAG review <sup>16</sup>	Current review
<i>Population</i>	Persons who may or may not have Lynch syndrome	All people newly diagnosed with colorectal cancer
<i>Intervention</i>	Any of the following (including combinations): <ul style="list-style-type: none"><li>• Strategies to identify Lynch syndrome in the population</li><li>• Strategies to manage Lynch syndrome in the population</li><li>• Strategies to manage patients in whom Lynch syndrome is identified</li></ul>	Microsatellite instability testing (with or without <i>BRAF</i> V600E mutation testing and with or without <i>MLH1</i> methylation testing)
<i>Comparator</i>	Current clinical practice (may or may not include efforts to identify Lynch syndrome)	At least one of: <ul style="list-style-type: none"><li>• Immunohistochemistry (with or without <i>BRAF</i> V600E mutation testing and with</li></ul>

		or without <i>MLH1</i> methylation testing)
		<ul style="list-style-type: none"> <li>• No testing</li> <li>• Direct constitutional MMR mutation testing</li> </ul>
<b>Outcomes</b>	Any of the following: <ul style="list-style-type: none"> <li>• Costs</li> <li>• Clinically relevant outcomes (e.g., life-years gained, QALYs, CRCs prevented)</li> <li>• Mutations detected</li> </ul>	Costs and health effects measured in life years or QALYs
<b>Study type</b>	Any of the following: <ul style="list-style-type: none"> <li>• Decision-analytic models (with or without a cost-effectiveness component)</li> <li>• Evaluations of cost-effectiveness within trials (including cost-effectiveness, cost-utility and cost-benefit studies; no requirement for randomisation)</li> <li>• Cost or resource use studies</li> <li>• Guidelines from national institutions, professional bodies and international bodies (including working groups)</li> </ul>	Any of the following: <ul style="list-style-type: none"> <li>• Decision analytic models</li> <li>• Economic evaluations within trials</li> </ul>

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

#### 4.1.2 Search strategy

The search strategy will include the following sources:

- Searching of electronic databases
  - MEDLINE (Ovid)
  - MEDLINE In-Process & Other Non-Indexed Citations (Ovid)
  - Embase (Ovid)
  - Web of Science (Thomson Reuters)
  - NHS EED (The Cochrane Library)
  - EconLit (EBSCO)
- The reviews by Snowsill et al.<sup>16</sup> and Grosse<sup>33</sup>
- Backward and forward citation chasing on included studies

Database searches will comprise population terms for Lynch syndrome or hereditary non-polyposis colorectal cancer (adapted from the previous PenTAG search terms), and intervention terms for MSI or IHC. A sample search strategy is given in Appendix 2.

A search filter will be used to restrict to economic evaluations which may meet the inclusion criteria, which will be adapted from the previous PenTAG search terms.

Searches will be date-limited to 2013 onwards (the previous PenTAG review had latest searches conducted on 5 February 2013) and will be restricted to English language only.

Computer assisted deduplication will be performed.

Studies will initially be screened according to title and abstract for potential inclusion. The screening will be done independently by at least two reviewers with experience of systematic reviews of economic evaluations. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary.

Full texts will be sought for any studies not excluded by title and abstract screening. These full texts will be screened independently by at least two reviewers for ultimate inclusion in the review. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary.

#### **4.1.3 Data extraction strategy**

Data will be extracted by one reviewer and checked by a second. Any disagreements will be resolved by discussion, with the involvement of a third reviewer if necessary.

The blank data extraction forms used in the previous PenTAG review will be used. The completed forms for studies included in the previous PenTAG review will be reused.

#### **4.1.4 Quality assessment strategy**

Quality appraisal will be conducted using the Drummond checklist,<sup>34</sup> since this was used in the previous PenTAG review. Quality appraisal will be performed by one reviewer and checked by a second.

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

#### **4.1.5 Methods of analysis/synthesis**

Where studies do not conduct a fully incremental cost-effectiveness analysis (e.g., if they perform a cost–consequences analysis), but it is possible to conduct such an analysis based on reported results, this will be done.

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

Narrative synthesis will be performed, supported by tabulation of study characteristics and results.

## **4.2 Development of a health economic model**

The health economic model previously developed by PenTAG will form the basis of the new economic evaluation. It has been validated through peer review in the NIHR HTA process and by the journal BMC Cancer.

The model will be updated with the following key changes:

- All newly-diagnosed colorectal cancer patients will be subject to testing for Lynch syndrome (in the base case) as opposed to only patients aged under 50 years;
- The set of diagnostic strategies under evaluation will be updated to reflect the final scope of this appraisal;
- The diagnostic performance of technologies will be re-estimated from the systematic review of diagnostic test accuracy (*Section 3*);

- Gynaecological surveillance for women diagnosed with Lynch syndrome will be included, as it is common in clinical practice (given that a previous structured review from PenTAG did not find evidence that surveillance was effective, this may be included solely as a cost);
- Costs of diagnostic tests, risk-reducing interventions and cancer treatment and follow-up will be updated to include new relevant data;
- Key model parameters will be updated to include new relevant data through the use of structured reviews.

#### **4.2.1 Model characteristics**

The economic model will adhere to the NICE reference case,<sup>35</sup> specifically:

- Cost-effectiveness results will be presented as incremental cost-effectiveness ratios of incremental costs to incremental QALYs;
- A lifetime time horizon will be used (to age 100 years);
- Costs will be included from a NHS and personal social services perspective;
- Direct health effects on patients and their relatives (offered testing for Lynch syndrome) will be included;
- A discount rate of 3.5% will be used for costs and QALYs.

#### **4.2.2 Data sources**

Diagnostic performance aspects of the model will be parameterised using the results of the systematic review of diagnostic test accuracy. Key parameters will also be identified through structured reviews, to ensure that they are not identified serendipitously, opportunistically or preferentially.<sup>36</sup> If these structured reviews identify existing recent systematic reviews then these may be utilised without further searching.

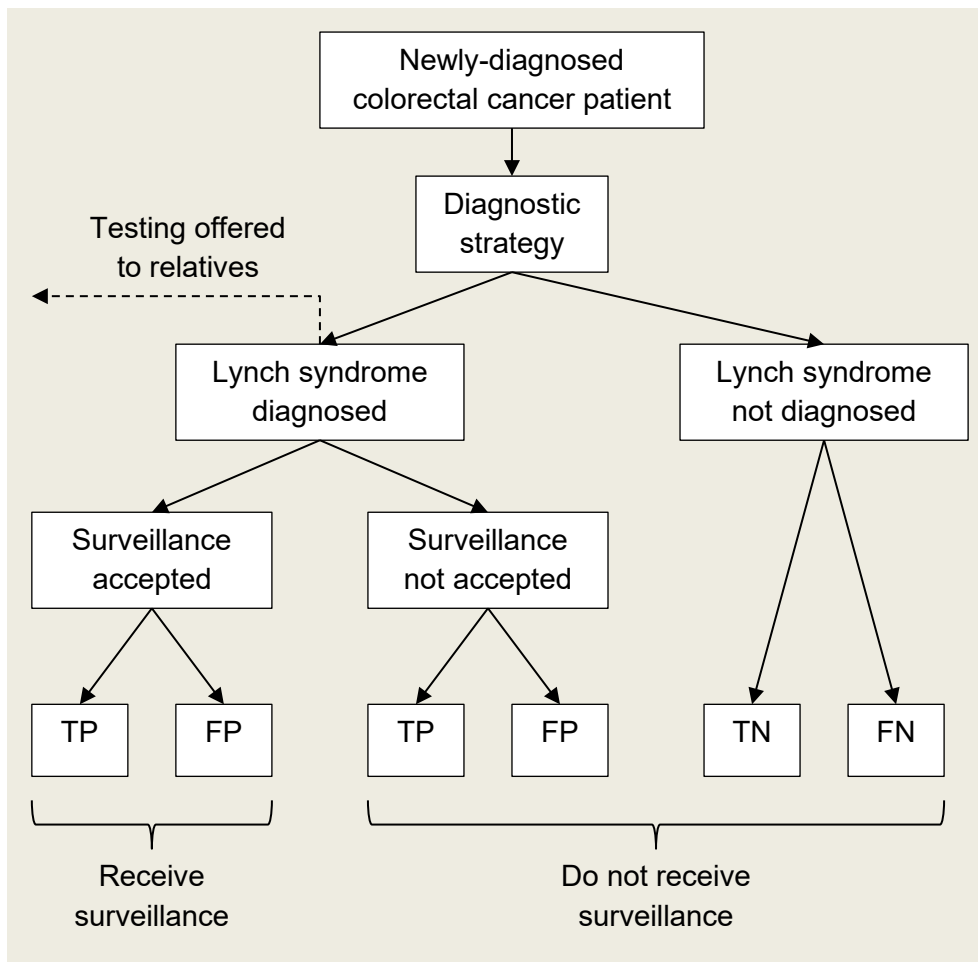
#### **4.2.3 Model structure**

The model will comprise two components:

- Diagnostic submodel (a decision tree);
- Outcomes submodel (an individual patient sampling model).

##### ***Diagnostic submodel***

The diagnostic submodel will simulate the paths of patients through the diagnostic pathway. It will incorporate information about the diagnostic performance of tests used in the pathway, as well as estimates of how many patients will accept different diagnostic tests and recommended colonoscopic surveillance (see *Figure 3*). It will also simulate diagnosis of Lynch syndrome in relatives of patients diagnosed with Lynch syndrome through cascade testing.



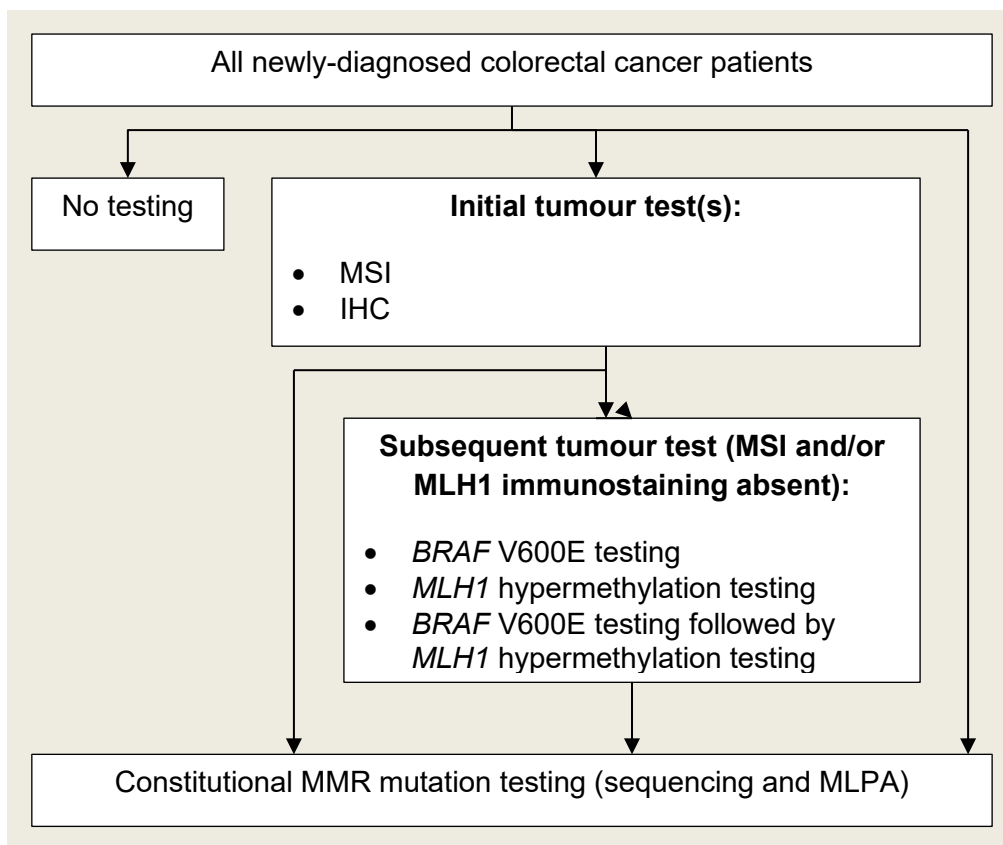
**Figure 3: Overview of diagnostic component of the health economic model**

The key outputs of the diagnostic submodel will be (for each diagnostic strategy):

- Number of patients diagnosed with Lynch syndrome, and of these:
  - Number of patients actually affected by Lynch syndrome;
  - Number of patients accepting surveillance;
- Number of relatives diagnosed with Lynch syndrome by cascade testing;
- Costs of diagnostic tests;
- Sensitivity and specificity.

The diagnostic strategies will be chosen to represent plausible clinical practice and to evaluate the cost-effectiveness of testing with MSI or IHC (see *Figure 4*). There will be a strategy of no testing (i.e., no attempt to identify Lynch syndrome), and a strategy of direct constitutional MMR mutation testing. There may be a strategy in which MSI and IHC are conducted in parallel since this may be clinical practice in some areas.

When IHC is not conducted as an initial tumour test for Lynch syndrome, IHC may be included as an adjunct to constitutional MMR mutation testing for a proportion of patients in order to aid interpretation.



**Figure 4: Diagnostic strategies in the health economic model**

### **Outcomes submodel**

The outcomes submodel will simulate the long-term impact of Lynch syndrome (diagnosed or undiagnosed) and risk-reducing measures on the risk of developing colorectal and endometrial cancer, and on overall survival.

Twenty-four separate patient groups will be simulated, according to:

- Sex (male/female);
- Individual with newly-diagnosed colorectal cancer or a relative;
- Truly affected or unaffected by Lynch syndrome;
- Lynch syndrome diagnosed and surveillance accepted *or* Lynch syndrome diagnosed and surveillance rejected *or* Lynch syndrome not diagnosed.

The outcomes submodel will be an event-driven individual patient sampling model. Individual patients will be simulated in isolation and then an average taken across a large sample of simulated individuals. Sampling individual patients enables full consideration of patient heterogeneity (e.g., a full age profile), but also introduces stochastic variability (the differences in outcomes for similar patients due to chance).

With the exception of colonoscopy (and colonoscopy-related morbidity and mortality), all events will be assumed to have a constant hazard rate within each year of the model.

Mortality events will be competing – once a mortality event occurs no further events can take place. *Table 4* details the events to be included in the model.

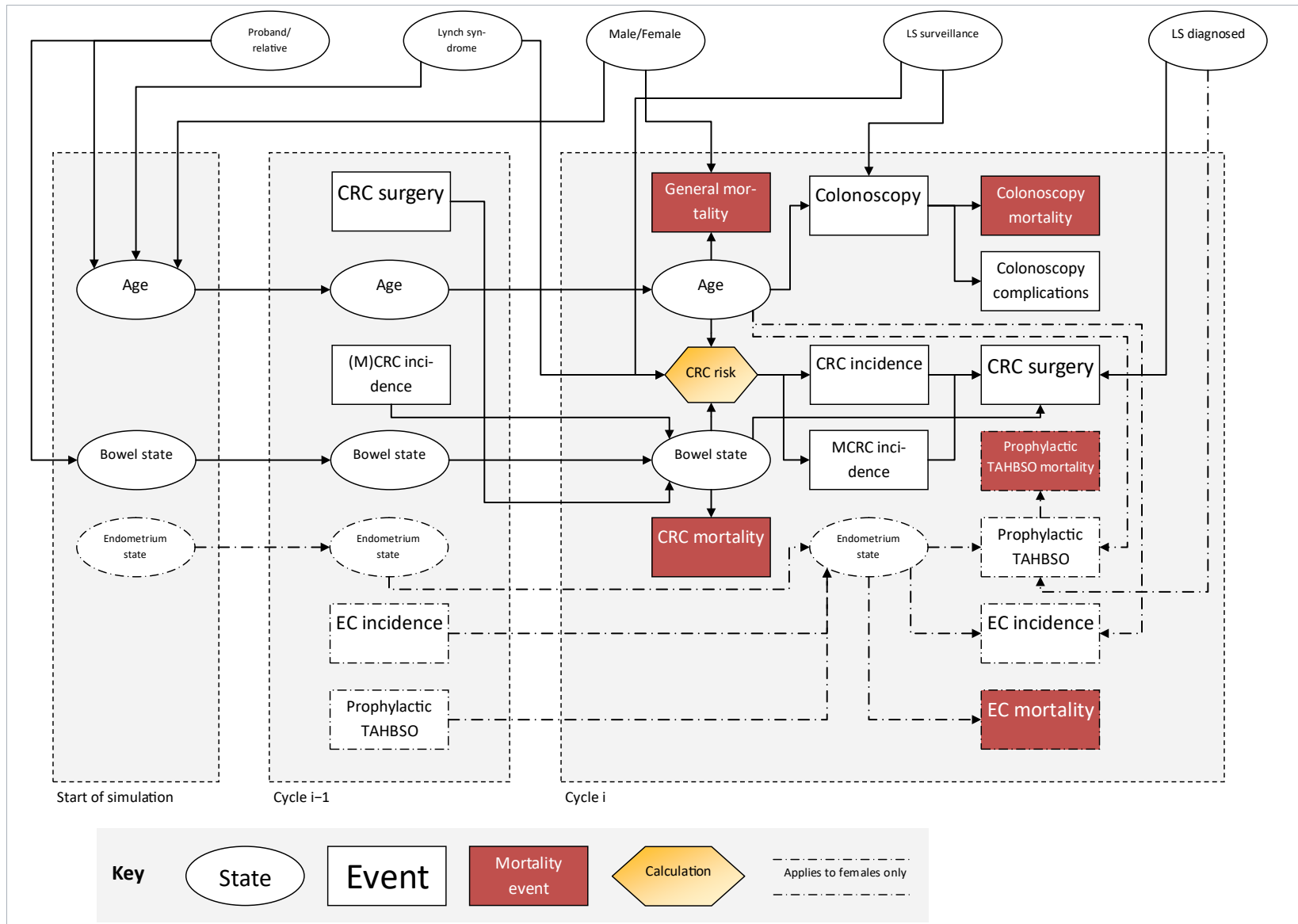
**Table 4: Events in the outcomes component of the health economic model**

Patient group	Competing events	Non-competing events
All patients	General mortality	
Patients undergoing LS surveillance (aged 25–75 years)	Mortality following colonoscopy	Colonoscopy Adverse events (includes bleeding and perforation) following colonoscopy
Patients with CRC (aged < 75 years)	Mortality following colonoscopy	Colonoscopy Adverse events (includes bleeding and perforation) following colonoscopy
Patients with CRC	CRC mortality	
Patients with an index CRC (without metachronous CRC)		Metachronous CRC incidence
Patients without CRC		CRC incidence
LS females without EC		EC incidence
LS females with EC	EC mortality	
Females diagnosed with LS without EC	Mortality following prophylactic H-BSO	Prophylactic H-BSO

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

Events in the model will affect the state of the patient, which will in turn affect the risk of particular events, as shown in *Figure 5*.

Costs in the outcomes submodel will be calculated from events, while QALYs will be calculated by assuming a utility profile which is dependent on age and whether the patient is affected by cancer and/or has had prophylactic surgery. Previously, a utility decrement was included for metastatic colorectal cancer in the base case.<sup>16</sup>



**Figure 5: Outcomes submodel**

Source: Previous PenTAG report<sup>16</sup>

#### **4.2.4 Exploration of uncertainty**

Uncertainty in the cost-effectiveness of different diagnostic strategies will be explored through one-way sensitivity analyses and scenario analyses (in which alternative parameter values for one or more parameters are substituted).

Due to the computational complexity of the individual patient sampling method in the outcomes submodel, uncertainty was not previously explored through probabilistic sensitivity analysis.

If time and resources permit, it may be possible to conduct a limited probabilistic sensitivity analysis (e.g., considering only uncertainty in diagnostic performance) or a full probabilistic sensitivity analysis using recent methodology as summarised in the NICE DSU Technical Support Document 15.<sup>37</sup>

## 5 Handling information from the companies

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As this appraisal has a clinical sponsor (the Royal College of Pathologists), we do not expect submissions from any companies. Notwithstanding, if any companies are entitled to make submissions through the NICE process, they will be considered if received by the EAG no later than 20 April 2016. Data arriving after this date will not be considered. If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Any 'commercial in confidence' data provided by a company and specified as such will be highlighted in **blue and underlined** in the assessment report (followed by an indication of the relevant company name, e.g., in brackets). Any 'academic in confidence' data provided and specified as such will similarly be highlighted in **yellow and underlined**.

## 6 Competing interests of authors

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All authors confirm that they have no potential competing interests.

## 7 Timetable/milestones

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<b>Milestone</b>	<b>Date to be completed</b>
Draft protocol	24 December 2015
Final protocol	27 January 2016
Progress report	27 April 2016
Draft assessment report	24 June 2016
Final assessment report	22 July 2016

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## Abbreviations

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AHRQ	Agency for Healthcare Research and Quality
CPA	Clinical Pathology Accreditation
CRC	Colorectal cancer
EC	Endometrial cancer
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
ESMO	European Society for Medical Oncology
FAP	Familial adenomatous polyposis
FDR	First-degree relative
H-BSO	Hysterectomy and bilateral salpingo-oophorectomy
HNPCC	Hereditary non-polyposis colorectal cancer (an alternative name for Lynch syndrome)
HTA	Health Technology Assessment
IHC	Immunohistochemistry
LS	Lynch syndrome
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
MSI	Microsatellite instability
NCI	National Cancer Institute
NICE DSU	National Institute for Health and Care Research Decision Support Unit
NIHR	National Institute for Health Research
PCR	Polymerase chain reaction
PenTAG	Peninsula Technology Assessment Group
QALY	Quality-adjusted life year
RCPATH	Royal College of Pathologists

## Appendices

### Appendix 1. Amsterdam I/II criteria, Bethesda and revised Bethesda guidelines

Table 5: Clinical criteria for Lynch syndrome

Criteria	Amsterdam	Amsterdam II	Bethesda	Revised Bethesda
	(All criteria must be met)		(At least one criteria must be met)	
<i>Individual clinical history</i>			<ul style="list-style-type: none"> <li>• Two LS-related cancers (including synchronous and metachronous)</li> <li>• CRC or EC &lt; 45 years</li> <li>• Right-sided CRC with undifferentiated pattern &lt; 45 years</li> <li>• Signet-ring-cell-type CRC &lt; 45 years</li> <li>• Adenomas &lt; 40 years</li> </ul>	<ul style="list-style-type: none"> <li>• CRC &lt; 50 years</li> <li>• Synchronous or metachronous LS-related tumours (regardless of age)</li> <li>• CRC with MSI-H histology &lt; 60 years</li> </ul>
<i>Family history</i>	<ul style="list-style-type: none"> <li>• Three or more relatives with CRC (one must be a FDR of the other two)</li> <li>• Two or more successive generations affected</li> <li>• One or more relatives diagnosed with CRC &lt; 50 years</li> </ul>	<ul style="list-style-type: none"> <li>• Three or more relatives with LS-related cancer (one must be a FDR of the other two)</li> <li>• Two or more successive generations affected</li> <li>• One or more relatives diagnosed with LS-related cancer &lt; 50 years</li> </ul>	<ul style="list-style-type: none"> <li>• Cancer and family meeting Amsterdam criteria</li> <li>• CRC and FDR with LS-related cancer and/or colorectal adenoma (one cancer diagnosed &lt; 45 years and adenoma diagnosed &lt; 40 years)</li> </ul>	<ul style="list-style-type: none"> <li>• CRC and FDR with LS-related cancer (one cancer diagnosed &lt; 50 years)</li> <li>• CRC and two or more first- or second-degree relatives with LS-related cancers (regardless of age)</li> </ul>
<i>Other</i>	<ul style="list-style-type: none"> <li>• FAP should be excluded</li> <li>• Tumours should be verified by pathologic examination</li> </ul>	<ul style="list-style-type: none"> <li>• FAP should be excluded for CRC cases</li> <li>• Tumours should be verified by pathologic examination</li> </ul>		

**Key:** CRC, colorectal cancer; EC, endometrial cancer; FAP, familial adenomatous polyposis; FDR, first-degree relative; HNPCC, hereditary non-polyposis colorectal cancer; LS, Lynch syndrome  
**Note:** LS-related cancers differ across criteria, see *Table 6*; MSI-H histology includes presence of tumour infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern

**Table 6: Lynch syndrome-related cancers according to different clinical criteria**

<b>Tumour</b>	<b>Amsterdam II</b>	<b>Bethesda</b>	<b>Revised Bethesda</b>
Colorectum	√	√	√
Endometrium	√	√	√
Small bowel	√	√	√
Ureter and renal pelvis	√	√	√
Ovaries		√	√
Stomach		√	√
Biliary tract		√	√
Pancreas			√
Brain (usually glioblastoma as seen in Turcot syndrome)			√
Sebaceous gland adenomas and keratocanthomas in Muir–Torre syndrome			√

## Appendix 2. Sample MEDLINE (Ovid) search strategies

### Sample clinical effectiveness search strategy:

1. (lynch\* adj3 syndrome).tw.
2. ((lynch\* adj3 famil\*) and (cancer\* or neoplasm\*)).tw.
3. or/1-2
4. ("Hereditary Nonpolyposis Colorectal Cancer" or "Hereditary Non-polyposis Colorectal Cancer").tw.
5. HNPCC.tw.
6. (((hereditary or inherited) adj3 (colon\* or colorectal\*)) and (cancer or neoplasm\*)).tw.
7. ((hereditary adj3 nonpolyposis) and (colon\* or colorectal\*)).tw.
8. ((hereditary adj3 non-polyposis) and (colon\* or colorectal\*)).tw.
9. ((hereditary adj3 (cancer or neoplasm\*)) and (colon\* or colorectal\*)).tw.
10. ((Familial adj3 Nonpolyposis) and (colon\* or colorectal\*)).tw.
11. ((Familial adj3 Non-polyposis) and (colon\* or colorectal\*)).tw.
12. (familial adj3 (colon\* or colorectal\*)).tw.
13. Colorectal Neoplasms, Hereditary Nonpolyposis/
14. or/4-13

15. (EPCAM? or MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2).tw.
  16. (colon\* or colorectal\* or lynch\* or HNPCC or hereditary).tw.
  17. 15 and 16
  18. Amsterdam criteria.tw.
  19. 3 or 14 or 17 or 18
  20. ((microsatellite adj3 instabilit\*) or (msi adj3 test\*)).tw.
  21. (Bethesda adj3 (marker\* or panel\*)).tw.
  22. (immunohistochemistry or (IHC adj3 test\*)).tw.
  23. ((MLH1 or MSH2 or MSH6 or hMSH2 or hMLH1 or hPMS2 or hMSH6 or PMS2) adj3 antibod\*).tw.
  24. ((BRAFV600E or "BRAF V600E") adj3 mutation\*).tw.
  25. (MLH1 adj3 (methylation or hypermethylation or "hyper methylation")).tw.
  26. exp Immunohistochemistry/
  27. or/20-26
  28. 19 and 27
  29. exp animals/ not humans.sh.
  30. 28 not 29
  31. limit 30 to (english language and yr="2006 -Current")
- Hits = 1372 (14<sup>th</sup> December 2015)

**Sample cost effectiveness search strategy:**

Lines 1-27 same as clinical effectiveness search;

28. exp Economics/
29. ec.fs.
30. economics, medical/
31. economics, nursing/
32. economics, pharmaceutical/
33. exp "economics, hospital"/
34. (economic\* or price or prices or pricing or priced or discount or discounts or discounted or discounting or ration\* or expenditure or expenditures or budget\* or afford\* or pharmaco-economic or pharmaco-economic\*).tw.
35. (cba or cea or cua).ti,ab.
36. exp "fees and charges"/
37. (fee or fees or charge\* or preference\*).tw.
38. (fiscal or funding or financial or finance).tw.
39. exp "costs and cost analysis"/

40. exp Health Care Costs/
41. cost\*.tw.
42. exp decision support techniques/
43. exp models, economic/
44. exp Statistical Model/
45. markov\*.tw.
46. markov chains/
47. monte carlo.tw.
48. monte carlo method/
49. (decision adj2 (tree\* or analy\* or model\*)).tw.
50. (survival adj3 analys\*).tw.
51. "deductibles and coinsurance"/
52. exp Health expenditures/
53. uncertain\*.tw.
54. uncertainty/
55. (quality adj3 life).tw.
56. quality of life/
57. value of life/
58. Quality-adjusted life years/
59. (qol\* or qoly or qolys or hrqol\* or qaly or qalys or qale or qales).tw.
60. (sensitivity analys\* or "willingness to pay" or quality-adjusted life year\* or quality adjusted life year\* or quality-adjusted life expectanc\* or quality adjusted life expectanc\*).tw.
61. utilit\*.tw.
62. valu\*.tw.
63. exp hospitalization/
64. or/28-63
65. 19 and 27 and 64
66. Animals/ not humans.sh.
67. 65 not 66
68. limit 67 to (english language and yr="2013 -Current")
69. Hits = 96 (14<sup>th</sup> December 2015)

### Appendix 3. Details of EAG and clinical advisors

Name	Institution	Role/expertise
EAG		

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Tristan Snowsill	PenTAG	Research Fellow; lead for cost-effectiveness (economic modelling and review of economic evaluations); project lead
Helen Coelho	PenTAG	Research Fellow; lead for systematic review of test accuracy
Nicola Huxley	PenTAG	Research Fellow; economic modelling; review of economic evaluations
Tracey Jones-Hughes	PenTAG	Research Fellow; systematic reviewer
Simon Briscoe	PenTAG	Information specialist
Martin Hoyle	PenTAG	Associate Professor of Health Technology Assessment; Director of PenTAG; economic modelling
Chris Hyde	PenTAG, Exeter Test Group, PenCLAHRC	Professor of Public Health and Clinical Epidemiology; public health physician; project guarantor
Sue Whiffin	ESMI	Senior administrator
Jenny Lowe	ESMI	Administrator; information officer
<i>Clinical advisors</i>		
Ian Frayling	Cardiff University, Cardiff & Vale University Local Health Board	Consultant in Genetic Pathology; Honorary Senior Clinical Research Fellow

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