National Institute for Health and Care Excellence

DIAGNOSTICS ASSESSMENT PROGRAMME

Evidence overview

KRAS mutation testing of tumours in adults with metastatic colorectal cancer

This overview summarises the key issues for the Diagnostics Advisory Committee's consideration. It includes a brief description of the topic, a description of the analytical structure and model, a discussion of the analytical difficulties, and a brief summary of the results. It is not a complete summary of the diagnostics assessment report, and it is assumed that the reader is familiar with that document. This overview contains sections from the original scope and the diagnostics assessment report, as well as referring to specific sections of these documents.

1 Background

1.1 Introduction

'KRAS mutation testing of tumours in adults with metastatic colorectal cancer' was selected by the Medical Technologies Advisory Committee (MTAC) for the Diagnostics Assessment Programme to develop recommendations on its use in the NHS. Multiple technologies and methods are available for performing KRAS mutation testing, including both CE marked tests and in-house laboratory techniques. Those identified during the scoping phase and included in the assessment are described in section 2.

The KRAS mutation tests are used as companion diagnostics for identifying patients with metastatic colorectal cancer who are most likely to respond to therapy with the epidermal growth factor receptor inhibiting monoclonal antibody, cetuximab. Patients with a KRAS wild-type tumour have been shown to gain benefit from treatment with cetuximab, in combination with

standard chemotherapy. However, patients with a KRAS mutant tumour do not respond to treatment with cetuximab so these patients would experience the toxic side effects of the drug unnecessarily. Therefore, patients with a KRAS mutant tumour will gain most benefit from being treated with standard chemotherapy alone.

The purpose of this assessment is to evaluate the clinical and cost effectiveness of using the different technologies and methods for KRAS mutation testing. Provisional recommendations on the use of these technologies and methods in the NHS will be formulated by the Diagnostics Advisory Committee at the Committee meeting on 4th September 2013.

It should be noted that NICE technology appraisal guidance 176 shows the epidermal growth factor inhibiting monoclonal antibody, cetuximab, to be cost-effective for the first line treatment of an optimised population of patients with metastatic colorectal cancer. The Diagnostics Assessment Programme evaluation will not be re-assessing the cost-effectiveness of cetuximab, but will be looking at the relative cost-effectiveness of the different techniques and tests included in the scope for evaluating KRAS mutation status.

1.2 The Condition

Colorectal cancer is the third most common cancer in the UK after breast and lung cancer, with approximately 40,000 new cases registered each year. Estimates of people presenting with stage IV metastatic colorectal cancer range from 20% to 55% of new cases. In addition, approximately 50 to 60% of patients who have undergone surgery for early stage colorectal cancer will eventually develop advanced disease and distant metastases, most commonly in the liver (typically presenting within 2 years of initial diagnosis). Colorectal cancer is the second most common cause of cancer death in the UK and the 5-year survival rate for metastatic colorectal disease is less than 7%. Of patients with advanced colorectal cancer, between 35% and 40% have mutations in the KRAS oncogene.

1.3 Diagnostic and care pathways

Diagnosis

NICE has produced a guideline on the diagnosis and management of colorectal cancer (2012, NICE clinical guideline 131). This guideline states that diagnostic investigations for people presenting to secondary care with suspected colorectal cancer include colonoscopy, flexible sigmoidoscopy followed by barium enema, or computed tomographic (CT) colonography.

If a lesion suspicious of cancer is detected, a biopsy is performed to confirm the diagnosis. All patients diagnosed with colorectal cancer are offered contrast-enhanced CT of the chest, abdomen and pelvis to estimate the stage of the disease. Further imaging may be considered if the CT scan shows metastatic disease only in the liver. The aim of further imaging is to identify:

• patients who have metastases suitable for resection

• patients who initially have unresectable metastases, but which can become resectable after a response to combination chemotherapy.

The aim of chemotherapy treatment in the second group of patients is to reverse initially unresectable metastatic colorectal cancer to resectable colorectal cancer. In this group of patients, European Society for Medical Oncology clinical practice guidelines for treatment of advanced colorectal cancer (2010) recommend establishing the KRAS status of the patient's tumour in order to determine the best treatment regimen. These guidelines do not stipulate which specific mutations should be analysed.

The KRAS status of a tumour is identified by analysing a section of resected tumour tissue, or sometimes a biopsy sample. The tissue is fixed in formalin and embedded in a block of paraffin (FFPE) for storage by the pathologist who also examines the histology and evaluates the tumour content of the sample. Macrodissection may be performed before DNA is extracted and mutation analysis is carried out to determine the KRAS status. If a sample is stored as a FFPE specimen for a long time this can lead to DNA degradation

which can result in a higher chance of failure when testing for KRAS mutations.

To minimise turnaround time, pathology guidelines recommend that in general, molecular diagnostic tests, such as a KRAS mutation test, should be ordered by the pathologist reporting on the histology of the tumour. However, this is not currently universal practice and often the decision to perform a KRAS mutation test is taken at the multidisciplinary team meeting.

The timing of the KRAS test can vary, with some clinicians preferring to test the KRAS status of patient's tumours at first diagnosis (reflex testing), potentially before the disease becomes metastatic, and other clinicians waiting until the cancer has progressed to metastatic disease (demand testing). If the KRAS status is tested early, the result is referred to if metastatic disease develops. Reflex testing avoids a potential delay of 2 to 4 weeks in starting treatment, which may occur if testing is performed once metastatic disease has developed. However, clinical opinion suggests that it is unlikely that a delay of 2 to 4 weeks would have a measurable impact in terms of disease progression and clinical outcomes.

There is suggestion that analysing multiple resection or biopsy samples from the same patient increases the chances of identifying a KRAS mutation due to heterogeneity between tumour sites. The evidence on this is conflicting, with studies claiming that testing a single site only will potentially misclassify between 2% and 10% of patients as KRAS wild-type.

Management/treatment

The KRAS status of a patient's tumour determines the best chemotherapy regimen. Patients with a KRAS wild-type tumour will gain most benefit from treatment with an epidermal growth factor receptor inhibiting monoclonal antibody in combination with standard chemotherapy. Patients with a KRAS mutant tumour will not respond to an epidermal growth factor receptor inhibiting monoclonal antibody, and therefore, will gain most benefit from receiving standard chemotherapy alone and avoiding the toxic side effects of

the antibody. In addition, the overall health and the preferences of the patient will likely influence the choice of treatment.

Chemotherapy

For patients with advanced or metastatic colorectal cancer, NICE clinical guideline 131 on the diagnosis and management of colorectal cancer (2012) recommends that one of the following sequences of chemotherapy is considered:

• Oxaliplatin in combination with infusional fluorouracil plus folinic acid (FOLFOX) as first line treatment then single agent irinotecan as second-line treatment.

• FOLFOX as first-line treatment then irinotecan in combination with infusional fluorouracil plus folinic acid (FOLFIRI) as second-line treatment.

• Oxaliplatin and capecitabine (XELOX) as first-line treatment then FOLFIRI as second-line treatment.

• Raltitrexed is considered only for patients with advanced colorectal cancer who are intolerant to fluorouracil and folinic acid, or for whom these drugs are not suitable.

Oral therapy with either capecitabine or tegafur with uracil (in combination with folinic acid) can also be considered as an option for the first-line treatment of metastatic colorectal cancer (NICE technology appraisal 61 on capecitabine and tegafur with uracil for metastatic colorectal cancer).

Biological agents

Cetuximab is a monoclonal antibody which inhibits the epidermal growth factor receptor. NICE technology appraisal guidance 176, Cetuximab for the first-line treatment of metastatic colorectal cancer recommends cetuximab in combination with FOLFOX or FOLFIRI, within its licensed indication, for the first-line treatment of patients with metastatic colorectal cancer in whom:

• The primary colorectal tumour has been resected or is potentially operable.

• The metastatic disease is confined to the liver and is unresectable.

• The patient is fit enough to undergo surgery to resect the primary colorectal tumour and to undergo liver surgery if the metastases become resectable after treatment with cetuximab.

Patients who meet the above criteria should receive treatment with cetuximab for no more than 16 weeks.

The European Medicines Agency marketing authorisation for cetuximab states that it is 'indicated for the treatment of patients with EGFR-expressing, KRAS wild-type metastatic colorectal cancer'. Therefore patients must have a KRAS mutation test and the tumour be identified as a wild-type KRAS before treatment with cetuximb can be considered.

Cetuximab (monotherapy or combination therapy) and bevacizumab (in combination with non-oxaliplatin chemotherapy) for the treatment of metastatic colorectal cancer after first-line chemotherapy are not recommended in NICE technology appraisal 242.

Ongoing care and support

All patients with primary colorectal cancer undergoing treatment with curative intent will have follow-up at a clinic visit 4 to 6 weeks after the potentially curative treatment (NICE clinical guideline 131). They will then have regular surveillance including:

• A minimum of two CT's of the chest, abdomen and pelvis in the first 3 years and

• Regular serum carcinoembryonic antigen tests (at least every 6 months in the first 3 years).

They will also have a surveillance colonoscopy at 1 year after initial treatment. If the result of this test is normal, they will have further colonoscopic follow-up after five years, and thereafter as determined by cancer networks.

1.4 The population

The population considered in this assessment is adults with previously untreated metastatic colorectal cancer in whom:

- The primary colorectal tumour has been resected or is potentially operable
- The metastatic disease is confined to the liver and is unresectable (confirmed by imaging as described in NICE clinical guideline 131)
- The patient is fit enough to undergo surgery to resect the primary colorectal tumour and to undergo liver surgery if the metastases become resectable after treatment with cituximab.

2 The technologies

Multiple methods are available for performing KRAS mutation testing, including both CE marked tests and in-house laboratory techniques.

Thirty UK based laboratories participated in the UK National External Quality Assessment Scheme (NEQAS) (2012-13, run 2, October 2012) for molecular genetic analysis of KRAS in colorectal cancer. These laboratories provided basic information on the methods they used to perform testing. In addition, UK laboratories have been contacted by NICE and asked to provide information on the methods they use for KRAS mutation testing. The methods used for KRAS mutation testing are presented in Table 1.

Table 1: Methods for KRAS mutation testing

Method		Number of laboratories using the method	
		NEQAS report (1)*	Lab contact†
Pyrosequencing		15	8
Sanger sequencing		6	1
Cobas KRAS mutation test		4	4
Therascreen KRAS kit	PCR Kit	3	1
	Pyro Kit		2
High resolution melt analysis		2	2
Real-time PCR		2	0
Mass spectrometry		1	0

* UK NEQAS pilot scheme 2012-2013, run 2. Thirty UK based laboratories participated in the scheme. Some laboratories used more than one method.

† NICE contact with laboratories October/November 2012. 15 laboratories provided information on methodologies used. Some laboratories used more than one method.

Based on information collected, the most popular technique for KRAS mutation testing is an in-house method of pyrosequencing codons 12, 13 and 61. Laboratories often have a back-up method to use if the sample has low tumour content, and the cobas KRAS Mutation Test is often used for this purpose. Several laboratories are also planning to switch to a next generation sequencing method over the next year.

CE marked tests

Therascreen KRAS RGQ PCR Kit (Qiagen)

The Therascreen KRAS RGQ PCR Kit is a CE marked real-time PCR assay for the detection of mutations in the KRAS gene. This kit has been given approval by the US Food and Drug Administration (FDA) to aid physicians in identifying patients with metastatic colorectal cancer for treatment with cetuximab. The Therascreen KRAS RGQ PCR Kit uses two technologies for the detection of mutations: ARMS (Amplification Refractory Mutation System) for mutation specific DNA amplification and Scorpions for detection of amplified regions. A real-time PCR instrument (Rotor-Gene Q 5-Plex HRM for consistency with CE-marking) is used to perform the amplification and to measure fluorescence.

The Therascreen KRAS RGQ PCR Kit is designed to detect the seven mutations in codons 12 and 13 of the KRAS gene (see Table 2). The limit of detection ranges from 0.77 to 6.43, depending on the mutation. The limit of detection is defined as the lowest amount of mutant DNA in a background of wild-type DNA at which a mutant sample will provide mutation-positive results in 95% of the test results.

An older version of this test exists – the Therascreen KRAS PCR Kit ('version 1') which was inherited from Qiagen's acquisition of DxS Ltd. This version also uses ARMS and Scorpions for the detection of KRAS mutations and is designed to detect the same KRAS mutations as the Therascreen KRAS RGQ PCR Kit ('version 2'). The 'version 2' kit differs from the 'version 1' kit in that it was reformulated using Qiagen reagents and was subject to a more rigorous validation process.

Therascreen KRAS Pyro Kit (Qiagen)

The Therascreen KRAS Pyro Kit is a CE marked test for the quantitative measurement of mutations the KRAS gene. The kit is based on pyrosequencing technology and consists of two assays: one for detecting

mutations in codons 12 and 13, and a second for detecting mutations in codon 61. The two regions are amplified separately by PCR, then amplified DNA is immobilised on Steptavidin Sepharose High Performance beads. Single-stranded DNA is prepared and sequencing primers added. The samples are then analysed on the PyroMark Q24 System. The KRAS Plug-in Report is recommended to analyse the results, however, the analysis tool within the pyrosequencer can also be used. The Therascreen KRAS Pyro Kit is designed to detect and quantify twelve mutations in codons 12, 13 and 61 of the KRAS gene (see Table 2). The limit of detection ranges from 1.0 to 3.5, depending on the mutation.

Cobas KRAS Mutation Test

The Cobas KRAS Mutation Test is a CE marked TaqMelt real-time PCR assay intended for the detection of 19 mutations in codons 12, 13 and 61 of the KRAS gene as presented in Table 2. The limit of detection ranges from 1.6 to 6.3, depending on the mutation. The assay uses DNA extracted from formalin-fixed paraffin-embedded tissue and is validated for use with the Cobas 4800 System. Mutation detection is achieved by PCR amplification of target DNA using labelled probes, followed by melting curve analysis. Data are analysed by the Cobas 4800 software and results are presented as 'mutation detected' (in codon 12/13, or codon 61, or both), or 'mutation not detected'.

KRAS StripAssay (ViennaLab)

The KRAS StripAssay is a CE marked test for the detection of mutations in the KRAS gene. There are two versions of the KRAS StripAssay: one is designed to detect 10 mutations in codons 12 and 13 of the KRAS gene; a second is designed to detect the same 10 mutations in codons 12 and 13 plus 3 mutations in codon 61 of the KRAS gene (see Table 2). The test procedure involves three steps: the DNA is first isolated from the specimen; PCR amplification is performed; the amplification product is then hybridised to a test strip containing allele-specific probes immobilised as an array of parallel

lines. Colour substrates are used to detect bound sequences which can then be identified with the naked eye or by using a scanner and software.

The assay is currently available for direct delivery to the UK, but the manufacturers plan to sell the product via a fixed UK distributor in the near future.

KRAS LightMix Kit (TIB MolBiol)

The KRAS LightMix Kit is a CE marked test designed for the detection and identification of 9 mutations in codons 12 and 13 of the KRAS gene (see Table 2). The first part of the test involves PCR amplification of the KRAS gene. In order to reduce amplification of the wild-type KRAS gene and therefore enrich the mutant KRAS gene, a wild-type specific competitor molecule is added to the reaction mix. This is called clamped mutation analysis. The second part of the test procedure involves melting curve analysis with hybridisation probes. The melting temperature is dependent on the number of mismatches between the amplification product and the probe, and allows the detection and identification of a mutation within the sample. The test is run on the LightCycler Instrument (Roche).

Table 2. Sequence variants that can be detected by the CE marked KRAS mutation test kits.

	Therascreen KRAS RGQ PCR kit	Therascreen KRAS Pyro	Cobas KRAS Mutation	KRAS StripAssay	KRAS LightMix kit
Codon 12	p.Gly12Ser	p.Gly12Ser	p.Gly12Ser	p.Gly12Ser	p.Gly12Ser
	p.Gly12Arg	p.Gly12Arg	p.Gly12Arg	p.Gly12Arg	p.Gly12Arg
	p.Gly12Cys	p.Gly12Cys	p.Gly12Cys	p.Gly12Cys	p.Gly12Cys
	p.Gly12Asp	p.Gly12Asp	p.Gly12Asp	p.Gly12Asp	p.Gly12Asp
	p.Gly12Ala	p.Gly12Ala	p.Gly12Ala	p.Gly12Ala	p.Gly12Ala
	p.Gly12Val	p.Gly12Val	p.Gly12Val	p.Gly12Val	p.Gly12Val
				p.Gly12Ile	
				p.Gly12Leu	
					p.Gly12Thr
Codon 13			p.Gly13Ser		
			p.Gly13Arg		
			p.Gly13Cys	p.Gly13Cys	p.Gly13Cys
	p.Gly13Asp	p.Gly13Asp	p.Gly13Asp	p.Gly13Asp	p.Gly13Asp
			p.Gly13Ala		
			p.Gly13Val		
Codon 61			p.Gln61Lys		
		p.Gln61Glu	p.Gln61Glu		
			p.Gln61Pro		
		p.Gln61Arg	p.Gln61Arg	p.Gln61Arg	
		p.Gln61Leu	p.Gln61Leu	p.Gln61Leu	
		p.Gln61His (1)	p.Gln61His (1)	p.Gln61His	
		p.Gln61His (2)	p.Gln61His (2)		

Laboratory based methods

Sanger sequencing

Sanger sequencing is used to detect all mutations within specific codons of the KRAS gene. Sequencing is a commonly used method; however, there is much variation in the detail of how the method is carried out. In general, after DNA is extracted from the sample it is amplified using PCR. The PCR product is then cleaned up and sequenced in both forward and reverse directions. The sequencing reaction uses dideoxynucleotides labelled with coloured dyes which randomly terminate DNA synthesis creating DNA fragments of various lengths. The sequencing reaction product is then cleaned up and analysed using capillary electrophoresis. The raw data are analysed using software to generate the DNA sequence. All steps are performed at least in duplicate to increase confidence that an identified mutation is real. It should be noted that sequencing only works well when viable tumour cells constitute at least 25% or more of the sample.

High resolution melt analysis

High resolution melt (HRM) analysis assays are designed to detect all mutations within specific codons of the KRAS gene. The DNA is first extracted from the sample and amplified using PCR. The HRM reaction is then performed. This involves a precise warming of the DNA during which the two strands of DNA ‘melt’ apart. Fluorescent dye which only binds to double stranded DNA is used to monitor the process. A region of DNA with a mutation will ‘melt’ at a different temperature to the same region of DNA without a mutation. These changes are documented as melt curves and the presence or absence of a mutation can be reported. The limit of detection for high resolution melt analysis is approximately 5% tumour DNA in a background of wild-type DNA (personal communication with laboratory staff).

Pyrosequencing

Pyrosequencing assays are designed to detect all mutations within specific codons of the KRAS gene. The process involves first extracting DNA from the

sample and amplifying it using PCR. The PCR product is then cleaned up before the pyrosequencing reaction. The reaction involves the sequential addition of nucleotides to the mixture. A series of enzymes incorporate nucleotides into the complementary DNA strand, generate light proportional to the number of nucleotides added and degrade unincorporated nucleotides. The DNA sequence is determined from the resulting pyrogram trace. Different laboratories may use slightly different primers for the amplification step, but the underlying methodology remains the same. The limit of detection for pyrosequencing is approximately 5-10% tumour DNA in a background of wild-type DNA (personal communication with laboratory staff).

MALDI-TOF mass spectrometry

MALDI-TOF (matrix assisted laser desorption ionization time-of-flight) mass spectrometry can be used to identify all mutations within selected codons in the KRAS gene. The process involves extracting DNA and amplifying it using PCR. An RNA intermediate is generated which is then cleaved and the fragments are separated based on mass by the MALDI-TOF mass spectrometer. This generates a 'fingerprint' of the DNA where each fragment is represented as a peak with a certain mass. The 'fingerprint' of the test sample is compared to the 'fingerprint' of the wild-type DNA using analysis software. A mutation would appear as a peak shift due to a change in the mass of a fragment caused by a base change. MALDI-TOF has a limit of detection of approximately 10% tumour DNA in a background of wild-type DNA.

Next generation sequencing

This method can be used to identify all mutations within specific codons of the KRAS gene. As with Sanger sequencing, there is much variation in the methodology used to perform next generation sequencing. The concept is similar to Sanger sequencing, however the sample DNA is first fragmented into a library of small segments that can be sequenced in parallel reactions. Some next generation sequencing methods use pyrosequencing methodology

rather than Sanger sequencing methodology. The limit of detection for next generation sequencing is approximately 5% tumour DNA in a background of wild-type DNA (personal communication with laboratory staff).

2.1 The Comparator

A range of methods for KRAS mutation testing are currently used in NHS laboratories. See the External Assessment Group's diagnostics assessment report (DAR) for details.

3 The evidence

3.1 Clinical effectiveness

The External Assessment Group conducted a systematic review of the evidence on the clinical effectiveness of the different methods used for KRAS mutation testing of tumours in adults with metastatic colorectal cancer. Supplementary evidence provided by the sponsors of the technologies is also included in the DAR. Additional data were obtained from an online survey of laboratories participating in the UK NEQAS pilot scheme for KRAS mutation testing. There were insufficient data for meta-analysis.

Details of the systematic review can be found starting on page 28 of the diagnostics assessment report. The objectives of the systematic review were to address three clinical effectiveness questions:

1. What is the technical performance of the different KRAS mutation tests, for example, proportion of tumour cells needed, limit of detection (minimum percentage mutation detectable against a background of wild-type DNA), failures, costs, turnaround time?
2. What is the accuracy (clinical validity) of KRAS mutation testing, using any test, for predicting response to treatment with cetuximab in combination with standard chemotherapy?
3. How do clinical outcomes from treatment with cetuximab in combination with standard chemotherapy and, where reported, from

treatment with standard chemotherapy, vary according to which test is used to select patients for treatment?

Separate inclusion criteria were developed for each of the three clinical effectiveness questions, summarised in table 3.

Table 3: Inclusion criteria

Question	What is the technical performance of the different KRAS mutation tests?	What is the accuracy of KRAS mutation testing, using any test, for predicting response to treatment with cetuximab in combination with standard chemotherapy?	How do outcomes from treatment with cetuximab in combination with standard chemotherapy and, where reported, from treatment with standard chemotherapy vary according to which test is used to select patients for treatment?
Participants:	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy. Patients who have been tested for KRAS mutation status.
Setting:	Secondary or tertiary care		
Interventions (index test):	Any commercial or in-house KRAS mutation test listed in Table 1	Any commercial or in-house KRAS mutation test listed in Table 1	First-line chemotherapy with cetuximab in combination with standard chemotherapy
Comparators:	Not applicable	Not applicable	Standard chemotherapy
Reference standard:	Not applicable	Response to treatment with cetuximab in combination with standard chemotherapy (e.g. progression free survival, objective response rate, disease control rate)	Not applicable
Outcomes:	Proportion tumour cells needed, failures, limit of detection, turnaround time, costs, expertise/logistics of test	Overall survival or progression free survival in patients whose tumours are KRAS mutant versus wild-type. Test accuracy – the number of true positive, false negative, false positive and true negative.	Progression free survival, overall survival, objective response rate, disease control rate
Study design:	To be addressed by survey; see below Publications from UK laboratories	RCTs (CCTs and cohort studies will be considered if no RCTs are identified)	RCTs (CCTs will be considered if no RCTs are identified)

Technical performance of KRAS mutation tests

No studies were identified from the systematic review which evaluated the technical performance of KRAS mutation tests.

There were 31 laboratories participating in the 2012-2013 UK NEQAS pilot scheme for KRAS mutation testing. Of these, 15 UK laboratories provided information that was included in the analysis by the External Assessment Group.

Pyrosequencing, using in-house methods, was the most commonly used KRAS mutation test, with nine laboratories using this approach. Although one of the laboratories using pyrosequencing stated that it was in the process of switching to high resolution melt analysis due to its quicker turnaround time. The Cobas KRAS Mutation Test was used by three laboratories, Sanger sequencing was used by two laboratories and only a single laboratory using the Therascreen KRAS Pyro Kit. One laboratory stated that it used high resolution melt analysis and direct sequencing.

More than half of the laboratories reported that KRAS mutation testing was performed on request (e.g. from a pathologist or oncologist) and only one laboratory reported routine testing of all colorectal cancer samples. There were no clear differences between tests in terms of batch size, turnaround time, number of failed samples or test costs. All laboratories reported a limit of detection for percentage mutation of ≤10%, except those using Sanger sequencing.

Accuracy of KRAS mutation testing

There was limited evidence on the accuracy of KRAS mutation testing. One study, the CELIM trial, reported sufficient data to allow estimation of the accuracy of a KRAS mutation test (Therascreen KRAS PCR kit) for predicting response to treatment with cetuximab plus standard chemotherapy. This study provided information on the extent to which KRAS mutation testing is able to discriminate between patients who will receive benefit from the addition of cetuximab to standard chemotherapy regimens and those who will not.

However, the study reported objective response and did not provide information on the value of the KRAS mutation test for predicting resection rate. To estimate accuracy of the test, the EAG assumed that the response to treatment was a result of the KRAS mutation status rather than any other factor affecting treatment response. The following definitions were used for the test accuracy statistics:

True positives	Patients with KRAS wild-type tumours who have a positive response to treatment with cetuximab plus standard chemotherapy
False positives	Patients with KRAS wild-type tumours who do not have a positive response to treatment with cetuximab plus standard chemotherapy
True negatives	Patients with KRAS mutant tumours who do not have a positive response to treatment with cetuximab plus standard chemotherapy
False negatives	Patients with KRAS mutant tumours who had a positive response to treatment with cetuximab plus standard chemotherapy

The sensitivity and specificity estimates for the Therascreen PCR Kit for predicting objective response (OR) were 74.6% (95% confidence interval [CI]: 62.1 to 84.5%), and 35.5% (95% CI: 19.2 to 54.6%) respectively.

Data from a second study, the COIN trial, allowed estimation of the accuracy of KRAS mutation tests (pyrosequencing and MALDI-TOF combined) for predicting response to treatment with cetuximab plus standard chemotherapy. Standard chemotherapy in this study did not match the inclusion criteria (some participants received XELOX) but, the data allowed the accuracy of the KRAS mutation tests for predicting the more clinically relevant outcome of potentially curative resection. The following definitions were used for the test accuracy statistics:

True positives	Patients with KRAS wild-type tumours who had a potentially curative resection following treatment with cetuximab plus, FOLFOX or XELOX
False positives	Patients with KRAS wild-type tumours who do not have a potentially curative resection following treatment with cetuximab, plus FOLFOX or XELOX
False negatives	Patients with KRAS mutant tumours who had a potentially curative resection following treatment with cetuximab, plus FOLFOX or XELOX
True negatives	Patients with KRAS mutant tumours who did not have a potentially curative resection following treatment with cetuximab plus FOLFOX or XELOX

The sensitivity and specificity estimates for the combination of pyrosequencing and MALDI-TOF for predicting potentially curative resection following treatment were 52.0% (95% CI: 31.3 to 72.2%) and 45.6% (95% CI: 37.0 to 54.3%), respectively.

Clinical effectiveness of cetuximab plus standard chemotherapy according to KRAS mutation testing

Four RCTs provided data on the clinical effectiveness of cetuximab plus standard chemotherapy compared to standard chemotherapy alone in patients with colorectal liver metastases whose tumours were KRAS wild-type. One trial (reported as a conference abstract) included only participants with unresectable colorectal liver metastases and no extra-hepatic metastases, whose tumours were KRAS wild-type. The other three trials, CRYSTAL, OPUS and COIN, included participants with metastatic colorectal cancer and conducted tumour KRAS mutation testing in a subgroup of these participants. Data were also reported for a smaller subgroup of participants whose metastases were confined to the liver and outcomes data were only reported for those with KRAS wild-type tumours.

Patient characteristics varied across studies. The CRYSTAL, OPUS AND COIN trials which reported subgroup data for patients with colorectal

metastases confined to the liver, were multi-centre studies conducted in Europe or, the UK and the Republic of Ireland. The subgroup data taken from these studies represented between 11% and 14% of the total study population. None of the studies reported separate participant characteristics for the relevant subgroup and none reported the criteria used to define unresectable liver metastases. For the larger KRAS wild-type subgroup, study participants were similar across the three studies. Further details of the study participants can be found in Appendix 2 of the DAR.

Two trials (CRYSTAL and OPUS) used the LightMix k-ras Gly12 assay (TIB MolBiol) to assess KRAS mutation status. The COIN trial used pyrosequencing together with MALDI-TOF mass spectrometry, and the remaining trial used pyrosequencing alone.

Clinical effectiveness results are presented in full starting on page 53 of the DAR, All four trials reported data on R0 resection rates in patients with colorectal metastases limited to the liver and KRAS wild type tumours and three of the four trials also reported objective response rate.

All of the studies reported the addition of cetuximab to standard chemotherapy was associated with an increase in the rate of R0 resections although this increase was only statistically significant in one trial (OR 4.57 [95% CI: 1.56 to 13.34]). All three studies which assessed objective response rate reported a statistically significant higher response rate for participants treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI: 1.49, 6.03) to 4.93 (95% CI: 1.42 to 17.06). No study reported an improvement in progression-free survival associated with the addition of Cetuximab to standard chemotherapy. One study reported a significant improvement in three year survival rates for participants treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy alone (OR 2.76, 95% CI: 1.12 to 6.26). Overall, there were no clear differences in treatment effect, regardless of which KRAS mutation test was used to select participants.

The median progression-free survival for participants with KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy was 11.8 months in the CRYSTAL trial and 11.9 months in the OPUS trial; the corresponding progression-free survival values in the standard chemotherapy groups were 9.2 months and 7.9 months. The median overall survival for participants with KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy was 27.8 months in the CRYSTAL trial and 26.3 months in the OPUS trial; the corresponding overall survival values in the standard chemotherapy groups were 27.7 months and 23.9 months.

3.2 Costs and cost effectiveness

3.2.1 Systematic review of cost effectiveness evidence

Four studies and one HTA report were included in the systematic review on the cost effectiveness of KRAS mutation testing. These are summarised on page 62 of the DAR.

In all of these publications, the ICERs for KRAS testing and treating only patients with KRAS wild type tumour status with cetuximab as compared to standard chemotherapy alone for all patients appear high.

3.2.2 Economic analysis

The EAG performed an economic analysis to assess the cost effectiveness of different methods of KRAS mutation testing to determine if standard chemotherapy plus cetuximab or standard chemotherapy alone is the appropriate treatment in patients with metastatic colorectal cancer whose metastases are confined to the liver and are unresectable. Standard chemotherapy regimens included FOLFOX and FOLFIRI. Each different KRAS testing method targets a different range of mutations and has different limits of detection (lowest proportion of mutation detectable in tumour cells). The exact combination of mutation type and level which will provide optimal treatment selection remains unclear. For this reason, assessment of test performance based on comparison with a conventional ‘reference standard’

was not possible and therefore, the EAG took a 'no comparator' approach to the analysis, which implies that the cost-effectiveness of each strategy will only be presented as compared to the next most cost-effective strategy.

Information on accuracy of tests (either based on objective response rate or tumour resection rate) to distinguish between patients with KRAS wild-type tumours and patients with KRAS mutant tumours with metastases confined to the liver, was only available for the Therascreen KRAS RGQ PCR Kit and pyrosequencing and MALDI-TOF. The COIN trial reported the testing of patients with both pyrosequencing and MALDI-TOF mass array, with a reported concordance of >99%. The EAG therefore assumed that for the economic evaluation, MALDI-TOF and pyrosequencing were equal; all results reported for pyrosequencing also applied to MALDI-TOF. However, survey data were only available for pyrosequencing, so the EAG only reported pyrosequencing in the results tables. No accuracy data were available for the other tests so comparisons were made based on the differences in technical performance and test costs retrieved from the online survey of NHS laboratories in England and Wales, whilst assuming a prognostic value equal to pyrosequencing across all tests. This assumption was made because there was no reliable evidence to model a difference in prognostic value for these tests.

Two analyses were performed by the EAG:

'Linked evidence' analysis

This analysis was used for all tests for which information on accuracy was available. The Therascreen® KRAS RGQ PCR Kit was compared with pyrosequencing based on objective response rates and resection rates for patients with a KRAS wild-type test result treated with cetuximab plus chemotherapy and for patients with a KRAS mutation test result treated with chemotherapy alone. Progression-free survival and overall survival after successful resection were assumed to be conditional on resection and treatment-independent. A major assumption underlying the use of these data

is that the differences in response rate and resection rate are solely due to the use of different KRAS mutation tests.

‘Assumption of equal prognostic value’ analysis

This analysis was used for all tests for which information on technical performance were available from the online survey. In this analysis, the different tests were compared based on test specific information on test failure rate only, whilst assuming equal prognostic value across tests. The equal prognostic value assigned was based on data for the pyrosequencing test (as this was the only test for which accuracy data were available on resection rates following treatment with chemotherapy, with and without cetuximab, for patients with initially inoperable liver metastases and both KRAS mutant and KRAS wild-type tumours). The following tests were included in this analysis:

- Cobas® KRAS Mutation Test (Roche Molecular Systems)
- Therascreen® KRAS RGQ PCR Kit (Qiagen)
- Therascreen® KRAS Pyro Kit (Qiagen)
- KRAS LightMix kit (TIB MolBiol)
- KRAS StripAssay (ViennaLab)
- High resolution melt analysis
- Pyrosequencing
- MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) Mass spectrometry
- Next generation sequencing
- Sanger sequencing

In order to ensure consistency between the modelling approach used in Technology Appraisal 176 and the assessment of the cost-effectiveness of different methods for KRAS mutation testing, the External Assessment Group received the electronic health economic model submitted by Merck Serono for Technology Appraisal 176. This model calculates the expected cost-effectiveness of cetuximab compared to chemotherapy for the first line treatment of metastatic colorectal cancer patients whose metastases are

confined to the liver and are unresectable and whose tumours are KRAS wild-type as tested with a pre-CE marked version of the LightMix KRAS Kit (TIB MolBiol). The External Assessment Group took into account amendments made by the Evidence Review Group (the academic group that assessed the Merck Serono health economic model on behalf of NICE) during the appraisal of cetuximab. This model was used to inform the development of a de novo model in which the long term consequences of using different KRAS mutation tests were assessed not only in patients with KRAS wild-type tumours, but also in patients with KRAS mutant tumours, or an unknown test result.

3.2.3 Model structure

A decision tree and a Markov model were developed to consider the long-term consequences of technical performance and accuracy of the different tests followed by treatment with cetuximab plus standard chemotherapy or standard chemotherapy alone in patients with metastatic colorectal cancer whose metastases are confined to the liver and are unresectable. The decision tree was used to model the test result (KRAS wild-type, KRAS mutant or unknown) and the accompanying treatment decision. In the model, patients with a KRAS wild-type tumour receive cetuximab plus standard chemotherapy and patients with a KRAS mutant tumour or an unknown KRAS status receive standard chemotherapy (i.e. FOLFOX). The decision tree is shown in figure 1.

The Markov model was used to estimate the long-term consequences in terms of costs and QALYs. The model with a cycle time of one week, and a lifetime time horizon (23 years were modelled using 1,200 cycles). Health states in the Markov model are (numbered according to NICE Technology Appraisal 176):

1) progression free first line - never operated
2) progressive disease second line - never operated
3) progressive disease second line – unsuccessful resection
4) survival after curative resection
5) progression free first line - unsuccessful resection
6) progressive disease third line – never operated
7) progressive disease third line – unsuccessful resection

8) dead

The Markov model is shown in figure 2.

Figure 1: Decision tree structure

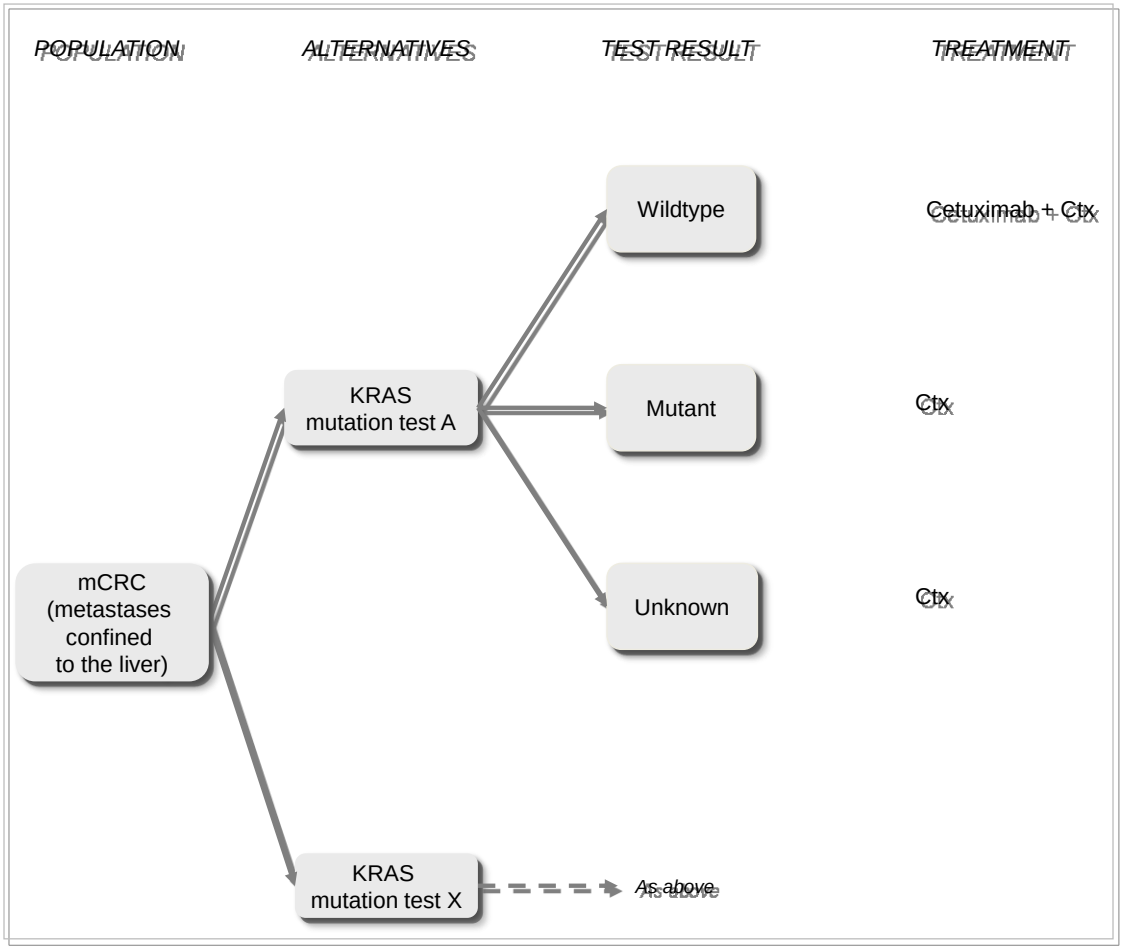
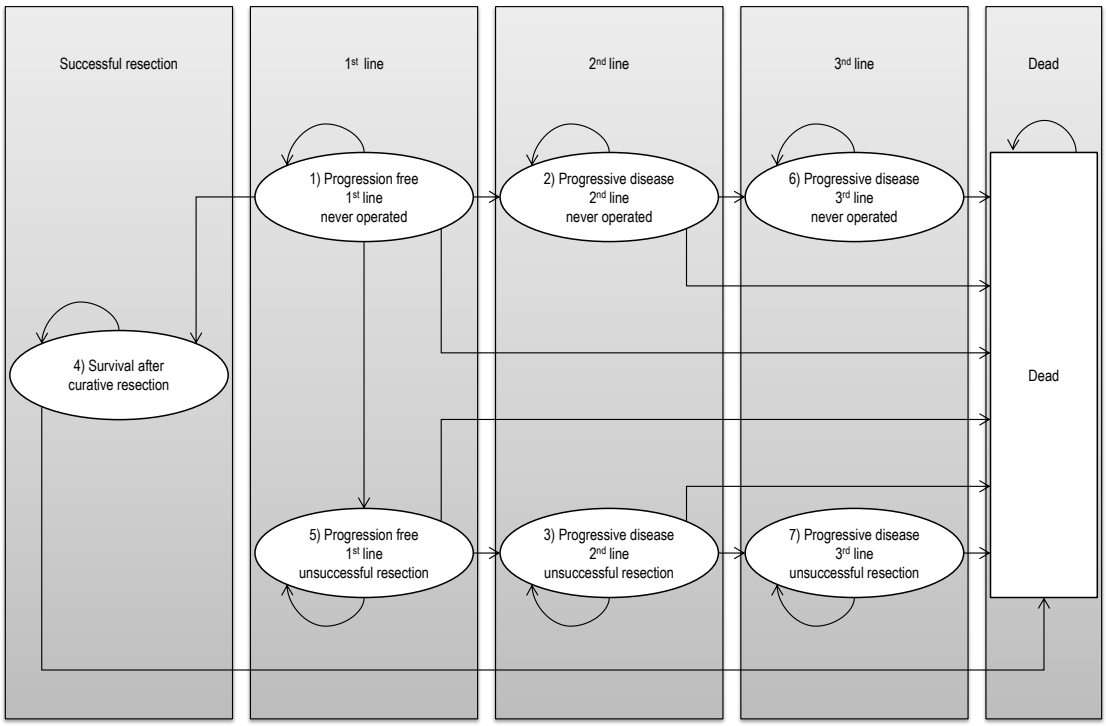


Figure 2: Markov model structure



3.2.4 Model inputs

Estimates for model input parameters were retrieved from NICE Technology Appraisal 176 and the manufacturer's submission for TA176, the assessment of the clinical effectiveness of different KRAS mutation tests (See section 3.2.2 of the DAR), and an online survey of NHS laboratories in England and Wales (See section 3.2.1 of the DAR).

3.2.5 Test results

The proportions of test failures in the laboratory for the KRAS mutation tests were based on the online survey of NHS laboratories in England and Wales. The proportions of KRAS wild-type and KRAS mutant test results were based on the estimated proportions of patients with KRAS wild-type tumours in the population (65.2% with standard error 0.8%), the test accuracy (sensitivity and specificity with objective response to cetuximab or resection rate as reference standard) and the proportion of patients with an unknown test result. The proportion of patients with an unknown test result was based on the proportions of patients with unknown tumour mutation status relative to the number of patients for whom a tissue sample was available in the clinical

trials. The proportion of patients with an unknown test result may be an over estimate as the clinical trials are unlikely to be representative of the true situation in current clinical practice. By contrast, the results of the online survey of laboratories in England and Wales are likely to provide an underestimation of the total proportion of patients with an unknown test result, as the laboratories may not have insight in the total proportion of pre-test failures (samples considered inadequate by the pathologist and therefore not sent to the laboratory). In the 'linked evidence' analysis, the proportion of unknowns was taken from the clinical trials. For the 'equal prognostic value' analysis, the proportion of unknowns for all tests was assumed to be equal to the pyrosequencing test.

Details of the input parameters used to calculate the proportion of patients with KRAS wild-type test result, unknown test result and mutant test results can be found starting on page 74 of the DAR.

Table 4: Probability of KRAS wild-type test result, unknown test result and KRAS mutant test result

Mutation test	Probability (se) of test result		
	Wild-type[a]	Unknown	Mutant[a]
Therascreen® KRAS RGQ PCR Kit	63.4% (4.7%)	10.8% (2.9%)	25.8% (4.4%)
Pyrosequencing	52.0% (0.8%)	1.7% (0.4%)	46.4% (0.8%)

se: standard error
[a] Standard error is based on probabilistic sensitivity analysis.

3.2.6 Resection rate

Patients who are in the 'progression-free first line – never operated' state can move to 'survival after successful resection', 'progression free first line – unsuccessful resection', progression free second line – never operated' or death, based on tumour resection rates, rate for failure of resection, and postoperative mortality. For patients with KRAS wild-type tumours, the resection rate after treatment with cetuximab and chemotherapy was used and the resection rate after treatment with chemotherapy alone was used for the remaining patients (KRAS mutant or unknown test results). The resection

rates reported and used in STA 176 for the chemotherapy-only strategy were calculated based on all patients (thus including patients with metastases not confined to the liver), and therefore probably are an underestimation of the true resection rate in the population with metastases confined to the liver. However, in the 'assumption of equal prognostic value' analysis the resection rate used was based on a trial including a population with liver-only metastases.

The resection failure rate was set at 5% and the probability of postoperative mortality was 2.8%, both consistent with STA176.

Table 5: Resection rates

Mutation test	Resection rate (se)[a,b]			Source
	Wild-type	Unknown	Mutant	
Therascreen® KRAS RGQ PCR Kit	0.433 (0.060)	0.092 (0.028)	0.092 (0.028)	CELIM,[52] Tournigand[68]
Pyrosequencing	0.149 (0.038)	0.132 (0.035)	0.132 (0.035)	COIN[54]

a: All resection rates were modelled using beta distributions.
b: In the 'equal prognostic value' analysis the response rate for pyrosequencing is used for all mutation tests.

3.2.7 Progression-free survival and overall survival

To ensure consistency with NICE Technology Appraisal 176, parametric survival models were obtained for patients without resection or with unsuccessful resection from this Technology Appraisal to estimate cycle-dependent progression free survival in the first and second line and overall survival in the first and third line. For patients with successful resection, parametric survival models were obtained from NICE Technology Appraisal 176 to calculate cycle-dependent progression free survival and overall survival probabilities (starting on page 78 of the DAR).

Progression free and overall survival in the first line for standard chemotherapy were based on data from the OPUS and CRYSTAL trials, respectively, and were estimated separately for patients treated with or without cetuximab. All progression free and overall survival probabilities for the first line are presented on page 77 of the DAR.

3.2.8 Adverse events

The occurrence of adverse events was assumed to be dependent on treatment and independent of tumour KRAS mutation status, i.e. occurrence of adverse events for patients with KRAS wild-type, KRAS unknown and KRAS mutant tumours were assumed to be equal among different test strategies. Consistent with STA 176, the occurrence of adverse events was only included in the model by incorporating the additional costs related to the adverse events based on the CRYSTAL and OPUS trials.

3.2.9 Health state utilities

Utility scores were in line with those used in NICE technology appraisal guidance 176 and are presented on page 81 of the DAR.

3.2.10 Resource use and costs

Resource use and costs were taken from NICE Technology Appraisal 176, with the exception of the KRAS test costs. These costs were based on the online survey of NHS laboratories in England and Wales. In the case of an unknown KRAS mutation status due to a pre-laboratory clinical failure, no test costs were taken into account. In the case of an unknown mutation status due to a technical failure within the laboratory full test costs were taken into account. This proportion was calculated based from the proportion of patients with an unknown mutation status as taken from the literature and the total proportion of technical failures in the laboratories as reported in the online survey. Other costs used in the analyses are presented on page 84 of the DAR.

3.2.11 Model analyses

The costs were evaluated from the perspective of the NHS and personal social services. The outcomes of the modelling were expressed as quality-

adjusted life years (QALYs). Both costs and outcomes were discounted using a 3.5% annual discount rate. The ICER represents the costs of an additional QALY gained and was used to estimate the cost-effectiveness of a strategy opposed to the next best alternative, as in the absence of a comparator strategy it was not possible to calculate ICERs relative to the comparator. All outcomes are based on probabilistic sensitivity analyses with 5,000 simulations using parameter distributions as presented in the DAR.

3.2.12 Key model assumptions

The main assumptions in the health economic analyses were:

1. The differences between objective response and resection rates for cetuximab plus chemotherapy versus chemotherapy alone reported in the trials are solely due to the different tests used (Therascreen KRAS RGQ PCR Kit and pyrosequencing) to distinguish between patients whose tumours are KRAS wild-type (and receive cetuximab plus chemotherapy) and patients whose tumours are KRAS mutant (and receive chemotherapy alone) ('linked evidence' analysis).

2. To calculate the sensitivity and specificity of the tests required to calculate the proportion of KRAS wild-type and KRAS mutant test results, patients tested as tumour KRAS wild-type were categorised as false positive if no objective response was observed (for Therascreen KRAS RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab, while patients were categorised as true positive if objective response was observed, or a liver resection was performed, respectively. Similarly, patients tested as tumour KRAS mutant were categorised as false negative if an objective response was observed (for Therascreen KRAS RGQ PCR Kit) or a liver resection was performed after treatment with cetuximab (for pyrosequencing) while patients were categorised as true negative if no objective response was observed or no liver resection was performed (both analyses).

3. Test accuracy based on objective response can be compared with accuracy based on resection rates.

4. The proportion of patients with unknown mutation status relative to the number of patients for whom a tissue sample was available in the trial provides a realistic approximation of the proportion of patients with an unknown test result in clinical practice (both analyses).

5. As the COIN trial tests for KRAS mutations with both pyrosequencing and MALDI-TOF with a reported concordance of >99%, it was assumed that the accuracy as derived from this trial and also the resection rates reported here apply to both pyrosequencing and MALDI-TOF. That is, all pyrosequencing results in this report also apply to MALDI-TOF.

6. The standard chemotherapy applied in the COIN-trial (FOLFOX or XELOX) is comparable to FOLFOX6 as used in the CELIM trial.

3.2.13 Sensitivity analyses

Two sensitivity analyses were performed for both the 'linked evidence' and the 'assumption of equal prognostic value' analyses:

- mortality in the second line was based on the average of first and third line mortality instead of background mortality as in STA 176.
- the proportion of unknown patients was based on the results of the online survey instead of the literature.

3.2.14 Results of cost-effectiveness analyses

As this economic evaluation takes a 'no comparator' approach, ICERs for each strategy are calculated as compared to the next most cost-effective strategy.

'Linked evidence' analysis

The 'linked evidence' analysis included two tests, pyrosequencing and the Therascreen KRAS RGQ PCR Kit. These tests were the only tests for which

evidence on test accuracy based on either resection rate or objective response was available. It should be noted that this analysis was based on a number of substantial assumptions, which are outlined in section 3.2.12.

Pyrosequencing results in the lowest total cost. The Therascreen KRAS RGQ PCR Kit is the more expensive but also more effective strategy, at an ICER of £17,019 per QALY gained (Table 7). The cost-effectiveness acceptability curve (see page 87 of the DAR) shows that for lower values of the threshold, pyrosequencing is to be preferred, and that the Therascreen KRAS RGQ PCR Kit is the most cost-effective option at thresholds of £17,000 and higher. The results of the sensitivity analyses (Table 7) do not differ substantially from the base case, in that the Therascreen KRAS RGQ PCR Kit is consistently more expensive and more effective than pyrosequencing, with ICERs ranging from £14,860 to £20,528 per QALY gained.

Table 6: Probabilistic results for 'linked evidence' analysis: base case and sensitivity analyses

Strategy	Costs	QALYs	Δ Costs	Δ QALYs	ICER
Base case					
Pyrosequencing*	£30,870	1.49			
Therascreen® KRAS RGQ PCR Kit	£33,995	1.67	£3,125	0.18	£17,019
Sensitivity analysis: mortality 2nd line based on average of 1st and 3rd line mortality					
Pyrosequencing*	£29,704	1.28			
Therascreen® KRAS RGQ PCR Kit	£33,132	1.51	£3,428	0.23	£14,860
Sensitivity analysis: unknowns from survey					
Pyrosequencing*	£30,714	1.48			
Therascreen® KRAS RGQ PCR Kit	£34,799	1.69	£4,085	0.20	£20,528

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry

‘Assumption of equal prognostic value’ analysis

The ‘assumption of equal prognostic value’ analysis includes all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales. In this analysis, the different tests were compared based on test specific information on test failure rate only, whilst assuming equal prognostic value across tests. The equal prognostic value assigned was based on data for the pyrosequencing test. In the base case and in the first sensitivity analysis, the total technical failure rate (pre-laboratory plus within laboratory technical failures) is assumed equal for all tests. In the base case, the average QALYs for all comparators were 1.48 (95% CI: 1.33 to 1.64). The total costs associated with the various testing strategies (Table 8) are highly similar. The same applies to the first sensitivity analysis (Table 8), costs are similar across strategies and average QALYs are equal by assumption at 1.28 (95% CI: 1.12 to 1.44).

Table 7: Probabilistic results for ‘assumption of equal prognostic value’ analysis, base case

	Costs (95% CI)	**Δ Costs** (95% CI)**
High resolution melt analysis	£30,857.09 (£27,079.58 - £34,736.14)	
Sanger sequencing	£30,857.09 (£27,079.58 - £34,736.14)	£0.00 (£0.00 - £0.00)***
Therascreen KRAS RGQ PCR Kit	£30,857.46 (£27,079.91 - £34,736.60)	£0.37 (£0.12 - £0.88)
Pyrosequencing*	£30,857.70 (£27,080.27 - £34,737.03)	£0.61 (£0.14 - £1.64)
Cobas KRAS Mutation Test	£30,857.99 (£27,080.25 - £34,737.14)	£0.91 (£0.23 - £2.28)

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
**Compared to least expensive comparator
***Costs were equal for High resolution melt analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).

Table 8: Probabilistic results for 'assumption of equal prognostic value', sensitivity analysis: mortality in second line based on average of first and third line

	Costs (95% CI)	Δ Costs** (95% CI)
High resolution melt analysis	£29,661.10 (£25,991.06 - £33,401.42)	
Sanger sequencing	£29,661.10 (£25,991.06 - £33,401.42)	£0.00 (£0.00 - £0.00)***
Therascreen KRAS RGQ PCR Kit	£29,661.47 (£25,991.81 - £33,401.80)	£0.37 (£0.12 - £0.85)
Pyrosequencing*	£29,661.71 (£25,992.12 - £33,401.81)	£0.61 (£0.14 - £1.59)
Cobas KRAS Mutation Test	£29,662.00 (£25,993.07 - £33,402.58)	£0.90 (£0.23 - £2.18)

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
**Compared to least expensive comparator
***Costs were equal for High resolution melt analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).

In the second sensitivity analysis the total technical failure rate is test specific, which impacts the proportion of patients with unknown (and therefore also wild-type and mutant) tumour KRAS status. All other input parameters, such as test costs and test accuracy, are still considered equal. The probabilistic results in Table 9 show that the Cobas KRAS Mutation test is the least costly and least effective strategy. High resolution melt analysis and Sanger sequencing have equal costs and effects and their ICER compared to the Cobas KRAS Mutation test is £69,815 per QALY gained. Pyrosequencing and the Therascreen KRAS RGQ PCR Kit are ruled out by extended dominance in this analysis. From the cost-effectiveness acceptability curve it is apparent that the Cobas KRAS Mutation test is the preferred strategy for all threshold values below £60,000 (see page 93 of the DAR).

Table 9: Probabilistic results for 'assumption of equal prognostic value' sensitivity analysis, unknowns based on survey

	Costs	QALYs	Comparator	Δ Costs	Δ QALYs	ICER
Cobas KRAS Mutation Test	£30,663	1.48				
Pyrosequencing*	£30,796	1.48	Cobas KRAS Mutation Test	£133.66	0.002	Extended dominance
Therascreen KRAS RGQ PCR Kit	£30,876	1.48	Pyrosequencing	£80.06	0.001	Extended dominance
High resolution melt analysis	£31,006	1.49	Cobas KRAS Mutation Test	£343.64	0.005	£69,815**
Sanger sequencing	£31,006	1.49	Cobas KRAS Mutation Test	£343.64	0.005	£69,815**

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
** High resolution melt analysis and Sanger sequencing were equally effective and equally expensive (as the survey indicated equal failure probabilities of 0% for both comparators).

4 Issues for consideration

The external assessment group found no clear evidence to suggest any differences between KRAS mutation testing techniques for any of the measures assessed (technical performance, accuracy for predicting response to treatment with cetuximab in combination with standard chemotherapy, or variation in clinical outcomes following treatment with cetuximab in combination with standard chemotherapy depending upon which method is used to classify patients as having KRAS wild-type tumours).

There were no published studies which evaluated the technical performance of any of the KRAS mutation tests. The survey of laboratories providing KRAS mutation testing indicated that in-house pyrosequencing methods, targeting KRAS mutations in codons 12, 13 and 61 and using self-designed primers were the most commonly used approach. The commercial KRAS mutation kits, Cobas KRAS Mutation Test and Therascreen KRAS Pyro Kit, were used by three laboratories and a single laboratory, respectively. Sanger sequencing was used in two laboratories. Reasons cited by respondents to the survey for

their choice of this technique were: proportion of tumour cells required; ease of use; cost; mutations covered; turnaround time; experience of pyrosequencing techniques available in the laboratory. There was no apparent association between test method and reason for choice.

More than half of the laboratories reported that KRAS mutation testing was performed on request (e.g. from a pathologist or oncologist) and only one laboratory reported routine testing of all colorectal cancer samples. There were no clear differences between tests in terms of batch size, turnaround time, number of failed samples or test costs. All laboratories reported a limit of detection for percentage mutation of ≤10%, except those using Sanger sequencing.

There was limited evidence on the accuracy of KRAS mutation testing. One study, the CELIM trial, reported sufficient data to allow estimation of the accuracy of a KRAS mutation test (Therascreen KRAS PCR kit) for predicting response to treatment with cetuximab plus standard chemotherapy. This study provided information on the extent to which KRAS mutation testing is able to discriminate between patients who will receive benefit from the addition of cetuximab to standard chemotherapy regimens and those who will not. However, the study reported objective response data and did not provide information on the value of the KRAS mutation test for predicting resection rate. To estimate accuracy of the test, the EAG assumed that the response to treatment was a result of the KRAS mutation status rather than any other factor affecting treatment response.

The positive predictive value, (70.2%, 95% CI: 57.7 to 80.7%) indicated that KRAS wild-type, as determined using the Therascreen KRAS PCR Kit, may be moderately predictive of tumour response. However, the negative predictive value (40.7%, 95% CI: 22.4 to 61.2%) could be interpreted as indicating that the presence of a KRAS mutation, as determined using the Therascreen KRAS PCR Kit, is a relatively poor predictor of non-response.

Data from a second study, the COIN trial, allowed estimation of the accuracy of KRAS mutation tests (pyrosequencing and MALDI-TOF combined) for

predicting response to treatment with cetuximab plus standard chemotherapy. Standard chemotherapy in this study did not match the inclusion criteria (some participants received XELOX) but, the data allowed the accuracy of the KRAS mutation tests for predicting the more clinically relevant outcome of potentially curative resection. The sensitivity and specificity estimates for the combination of pyrosequencing and MALDI-TOF for predicting potentially curative resection following treatment were 52.0% (95% CI: 31.3 to 72.2%) and 45.6% (95% CI: 37.0 to 54.3%), respectively. The positive and negative predictive values derived from these data were 14.9% (95% CI: 8.9 to 23.9%) and 83.9% (95% CI: 73.8 to 90.5%), respectively. This could be interpreted as indicating that a tumour which is defined as KRAS wild-type by this method is a poor predictor of resectability following treatment with cetuximab plus standard chemotherapy, where as the presence of a KRAS mutation is a good predictor of non-response (tumour remaining unresectable after treatment. However, it should be noted that any apparent differences in the ability of KRAS mutation tests to predict response to treatment could be caused by other differences between the two trials (e.g. participant characteristics, treatment regimens).

Four RCTs compared the clinical effectiveness of cetuximab plus standard chemotherapy compared to standard chemotherapy alone, in patients with colorectal liver metastases whose tumours were KRAS wild-type. All of the RCTs reported data on patients with colorectal cancer metastases which were confined to the liver. Each trial used a different chemotherapy regimen and there was no substantial evidence to indicate a significant difference in treatment effect depending on which of the KRAS mutation tests was used (LightMix k-ras Gly12, pyrosequencing and MALDI-TOF mass array for mutations in codons 12, 13 and 61, or pyrosequencing for KRAS mutations in codons 12 and 13).

The three studies which assessed objective response rate all reported a statistically significant higher response rate for participants treated with cetiximab plus standard chemotherapy compared to those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI: 1.49, 6.03) to

4.93 (95% CI: 1.42 to 17.06). All four studies reported that the addition of cetuximab to standard chemotherapy was associated with an increase in the rate of R0 resections following treatment. However, it should be noted that the only trial to report a statistically significant treatment effect for R0 resection rate used pyrosequencing to identify KRAS mutations in codons 12 and 13 only. This was also the only trial in which all participants had CRC metastases which were limited to the liver.

The review of evidence on the cost-effectiveness of KRAS mutation testing to determine appropriate treatment (cetuximab plus chemotherapy or chemotherapy alone) identified four studies and one HTA report. In all of these publications, the ICERs for KRAS testing and treating only patients with KRAS wildtype tumour status with cetuximab as compared to standard chemotherapy alone for all patients appeared high.

The EAG performed an economic analysis to assess the cost effectiveness of different methods of KRAS mutation testing to determine if standard chemotherapy plus cetuximab or standard chemotherapy alone is the appropriate treatment in patients with metastatic colorectal cancer who metastases are confined to the liver and are unresectable. Two analyses were performed: 'linked evidence', and 'assumption of equal prognostic value'. All analyses took a 'no comparator' approach.

In the 'linked evidence' analysis, the Therascreen KRAS RGQ PCR Kit was compared to pyrosequencing, using the available objective response and resection rate, respectively. The results of this analysis suggested that the Therascreen KRAS RGQ PCR Kit was more costly and more effective than pyrosequencing at an ICER of £17,019 per QALY gained. Sensitivity analyses did not show substantial differences compared to the base case. The key driver behind the outcome was the difference in resection rate between treatment with and without cetuximab and the proportion of patients with KRAS wild-type, KRAS mutant, and unknown tumours. This was determined by test accuracy and therefore, was dependent on objective response rate (for Therascreen KRAS RGQ PCR Kit) or resection rate (for pyrosequencing). As

detailed in section 3.2.12, this analysis was based on a number of substantial assumptions. Two key assumptions that are likely to significantly impact the model results are:

1. The differences between objective response and resection rates for the different treatment strategy (with or without cetuximab) reported in the trials are solely due to the different KRAS mutation tests used (Therascreen KRAS RGQ PCR Kit and pyrosequencing).

2. To calculate the proportion of KRAS wild-type and KRAS mutant test results, patients tested as tumour KRAS wild-type were categorised as false positive if no objective response was observed (for Therascreen KRAS RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab. Patients were categorised as true positive if objective response was observed, or a liver resection was performed. Similarly, patients tested as tumour KRAS mutant were categorised as false negative if an objective response was observed (for Therascreen KRAS RGQ PCR Kit) or a liver resection was performed after treatment with cetuximab (for pyrosequencing) while patients were categorised as true negative if no objective response was observed or no liver resection was performed (both analyses).

The 'assumption of equal prognostic value' analysis included all tests for which information on technical performance were available from the online survey. In this analysis, the different tests were compared based on test specific information on test failure rate only, whilst assuming equal prognostic value across tests. The equal prognostic value assigned was based on data for the pyrosequencing test (as this was the only test for which accuracy data were available on resection rates following treatment with chemotherapy, with and without cetuximab, for patients with initially inoperable liver metastases and both KRAS mutant and KRAS wild-type tumours).

The results of the 'assumption of equal prognostic value' analysis indicated that the strategies were almost equal. The first sensitivity analysis confirmed this. The second sensitivity analysis, for which the rate of unknown mutation

status was taken from the survey instead of the literature, did not assume equal effectiveness among all tests. The results showed that the Cobas KRAS Mutation Test was the least expensive and least effective strategy, and that Sanger sequencing and high resolution melt analysis were most costly and most effective at an ICER of £69,815 per QALY gained compared to the Cobas KRAS Mutation Test. The other two strategies included in this analysis, the Therascreen KRAS RGQ PCR Kit and pyrosequencing, were ruled out by extended dominance.

It should be noted that the uncertainty resulting from the assumptions made in the economic analyses was not parameterised in the model and is therefore, not reflected in the probabilistic sensitivity analyses or in the cost-effectiveness acceptability curves.

5. Equality considerations

NICE is committed to promoting equality of opportunity, eliminating unlawful discrimination and fostering good relations between people with particular protected characteristics and others.
The target group for the technology (adults with metastatic colorectal cancer) falls within the provisions of the Equality Act 2010 from the point at which a diagnosis of cancer has been made.
The highest incidence rates of colorectal cancer are in older men and women, with almost three-quarters of cases occurring in people aged 65 years and over.

6 Implementation

Any laboratories testing for KRAS mutation status, either using a CE marked or a non-CE marked technique must show compliance with an accredited external quality assurance scheme such as those provided by UK NEQAS and the European Molecular Genetics Quality Network (EMQN). Furthermore, laboratories should be accredited in the discipline of molecular diagnostics and any test used should be validated.

7 Authors

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August 2013

Appendix A: Sources of evidence considered in the preparation of the overview

A The diagnostics assessment report for this assessment was prepared by Kleijnen Systematic Reviews Ltd:

Westwood ME, van Asselt ADI, Ramaekers BLT, Whiting P, Joore MA, Armstrong N, Noake C, Ross J, Severens JL, Kleijnen J. KRAS mutation testing in adults with metastatic colorectal cancer: a systematic review and cost-effectiveness analysis. A Diagnostic Assessment Report. Kleijnen Systematic Reviews Ltd, 2013.

B The following organisations accepted the invitation to participate in this assessment as stakeholders. They were invited to attend the scoping workshop and to comment on the diagnostics assessment report.

I Manufacturers/sponsors:

- Qiagen Ltd.
- Roche Molecular Systems, Inc.
- Randox Laboratories Ltd.
- ViennaLab Diagnostics GmbH
- Merck Serono S.A
- TIB MOLBIOL GmbH

II Professional/specialist and patient/carer groups:

- All Wales Genetics Laboratory
- Association of Coloproctologists of Great Britain & Ireland (ACPGBI)
- Birmingham Women's NHS Foundation Trust
- Bladder & Bowel Foundation
- Bowel Cancer UK
- Bristol Royal Infirmary
- Cheshire & Merseyside Regional Genetics Laboratories
- Department of Health
- European Molecular Genetics Quality Network (EMQN)
- Guy's & St. Thomas NHS Foundation Trust
- Lab21 Ltd.
- Leeds Institute of Molecular Medicine
- Mount Vernon Cancer Centre
- NHS Lothian
- Nottingham University Hospitals NHS Trust
- Oxford BRC/NHS Molecular Diagnostic Centre
- Queen Elizabeth Hospital Birmingham
- Queen’s University Belfast
- Royal College of Nursing
- Royal College of Pathologists
- Royal College of Physicians
- Royal Devon and Exeter NHS Foundation Trust
- Sheffield Diagnostic Genetics Service

- St. Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust
- The Royal Marsden NHS Foundation Trust
- UK NEQAS for Molecular Genetics
- University Hospitals Birmingham NHS Foundation Trust
- Wessex Regional Genetics Laboratory