

NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE

Diagnostics Assessment Programme

KRAS mutation testing of tumours in adults with metastatic colorectal cancer

Final scope

January 2013

1 Introduction

The Medical Technologies Advisory Committee identified 'KRAS mutation testing of tumours in adults with metastatic colorectal cancer' as potentially suitable for evaluation by the Diagnostics Assessment Programme on the basis of a briefing note. The final scope was informed by discussions at the scoping workshop held on 3rd December and the assessment subgroup meeting held on 18th December 2012 (attendees listed in appendix F). A glossary of terms and a list of abbreviations are provided in appendices A and B.

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.

2 Description of the technologies

This section describes the properties of the diagnostic technologies based on information provided to NICE by manufacturers and experts advisers. NICE has not carried out an independent evaluation of these descriptions.

2.1 Purpose of the medical technology

KRAS mutation testing is indicated in adults with metastatic colorectal cancer, where metastases are confined to the liver and are unresectable. The presence or absence of certain KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations can affect the response of tumours to therapies. Studies have shown that in patients without KRAS mutations in their tumours (KRAS wild-type), treatment with epidermal growth factor inhibiting monoclonal antibodies leads to improved patient outcomes compared to treatment with standard chemotherapy. In patients with KRAS mutations in their tumours, treatment with epidermal growth factor inhibiting monoclonal antibodies has no beneficial effect compared to treatment with standard chemotherapy.

The purpose of KRAS mutation testing is to screen the tumours of adults with metastatic colorectal cancer for KRAS mutations to identify tumours with a KRAS mutation which will not benefit from treatment with epidermal growth factor inhibiting monoclonal antibodies, such as cetuximab.

2.2 Product properties

Multiple methods are available for performing KRAS mutation testing, including both CE marked tests and in-house laboratory techniques. These are detailed in sections 2.2.1 to 2.2.10.

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.

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.

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	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.

CE marked tests

2.2.1 Therascreen KRAS RGQ PCR Kit (Qiagen)

The therascreen KRAS RGQ PCR Kit is a CE marked real-time PCR assay for the detection of seven mutations in codons 12 and 13 of 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 (2).

The therascreen KRAS RGQ PCR Kit is designed to detect the mutations listed in 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 (2).

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. The therascreen KRAS PCR Kit ('version 1') is validated

for use on the Roche LightCycler 480 Real-Time PCR System and the Applied Biosystems 7500 Real-Time PCR System (3).

Table 2: Sequence variants that can be detected by the thescreen KRAS RGQ PCR Kit

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
12	c.34G>A	p.Gly12Ser	p.G12S
	c.34G>C	p.Gly12Arg	p.G12R
	c.34G>T	p.Gly12Cys	p.G12C
	c.35G>A	p.Gly12Asp	p.G12D
	c.35G>C	p.Gly12Ala	p.G12A
	c.35G>T	p.Gly12Val	p.G12V
13	c.38G>A	p.Gly13Asp	p.G13D

Scoping searches identified one published study; an end-to-end study using the thescreen KRAS RGQ PCR Kit to retrospectively identify colorectal cancer patients treated with or without cetuximab (4). This study was the basis of approval by the FDA. A comparison of the kit to bidirectional sequencing is also detailed in the product handbook (2). There are however many published studies of 'version 1' of the kit (thescreen KRAS PCR Kit).

2.2.2 Therascreen KRAS Pyro Kit (Qiagen)

The thescreen KRAS Pyro Kit is a CE marked test for the quantitative measurement of twelve mutations in codons 12, 13 and 61 of 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 (5).

The thescreen KRAS Pyro Kit is designed to detect and quantify the mutations listed in Table 3. The limit of detection ranges from 1.0 to 3.5, depending on the mutation (5). One conference abstract on the thescreen KRAS Pyro kit was identified during scoping searches (6). In addition, evidence on in-house pyrosequencing for the detection of KRAS mutations is likely to be transferable to the thescreen KRAS Pyro Kit.

Table 3: Sequence variants that can be detected by the thescreen KRAS Pyro Kit

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
12	c.34G>A	p.Gly12Ser	p.G12S
	c.34G>C	p.Gly12Arg	p.G12R
	c.34G>T	p.Gly12Cys	p.G12C
	c.35G>A	p.Gly12Asp	p.G12D
	c.35G>C	p.Gly12Ala	p.G12A
	c.35G>T	p.Gly12Val	p.G12V
13	c.38G>A	p.Gly13Asp	p.G13D
61	c.181C>G	p.Gln61Glu	p.Q61E
	c.182A>G	p.Gln61Arg	p.Q61R
	c.182A>T	p.Gln61Leu	p.Q61L
	c.183A>C	p.Gln61His (1)	p.Q61H (1)
	c.183A>T	p.Gln61His (2)	p.Q61H (2)

2.2.3 Cobas KRAS Mutation Test (Roche Molecular Systems)

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 4. 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 (7). 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'. Two published studies (8;9) and six conference abstracts (10-15) comparing the cobas KRAS mutation to another method for detecting KRAS mutations were identified during scoping searches.

Table 4: Sequence variants that can be detected by the cobas KRAS Mutation Test

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
12	c.34G>A	p.Gly12Ser	p.G12S
	c.34G>C	p.Gly12Arg	p.G12R
	c.34G>T	p.Gly12Cys	p.G12C
	c.35G>A	p.Gly12Asp	p.G12D
	c.35G>C	p.Gly12Ala	p.G12A
	c.35G>T	p.Gly12Val	p.G12V

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
13	c.37G>A	p.Gly13Ser	p.G13S
	c.37G>C	p.Gly13Arg	p.G13R
	c.37G>T	p.Gly13Cys	p.G13C
	c.38G>A	p.Gly13Asp	p.G13D
	c.38G>C	p.Gly13Ala	p.G13A
	c.38G>T	p.Gly13Val	p.G13V
61	c.181C>A	p.Gln61Lys	p.Q61K
	c.181C>G	p.Gln61Glu	p.Q61E
	c.182A>C	p.Gln61Pro	p.Q61P
	c.182A>G	p.Gln61Arg	p.Q61R
	c.182A>T	p.Gln61Leu	p.Q61L
	c.183A>C	p.Gln61His (1)	p.Q61H (1)
	c.183A>T	p.Gln61His (2)	p.Q61H (2)

2.2.4 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 (Table 5). 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 (22).

Four published studies and two conference abstracts comparing the KRAS StripAssay to other methods for detecting KRAS mutations were identified during early scoping (23-28). 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.

Table 5: Sequence variants that can be detected by the KRAS StripAssay

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
12	c.34G>A	p.Gly12Ser	p.G12S
	c.34G>C	p.Gly12Arg	p.G12R
	c.34G>T	p.Gly12Cys	p.G12C
	c.35G>A	p.Gly12Asp	p.G12D
	c.35G>C	p.Gly12Ala	p.G12A
	c.35G>T	p.Gly12Val	p.G12V
	c.[34G>A; 35G>T]	p.Gly12Ile	p.G12I
	c.[34G>C; 35G>T]	p.Gly12Leu	p.G12L
13	c.37G>T	p.Gly13Cys	p.G13C
	c.38G>A	p.Gly13Asp	p.G13D
61	c.182A>G	p.Gln61Arg	p.Q61R
	c.182A>T	p.Gln61Leu	p.Q61L
	c.183A>T	p.Gln61His	p.Q61H

2.2.5 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 (Table 6). 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) (16).

One published study and one conference abstract comparing the KRAS LightMix Kit with other methods for detecting KRAS mutations were identified during scoping searches (17;18). In addition, a pre-CE marked version of the kit was used in three end-to-end studies, which tested colorectal cancer patients for KRAS mutations and followed them through treatment with chemotherapy with or without cetuximab (19-21).

Table 6: Sequence variants that can be detected by the KRAS LightMix Kit

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-letter code
12	c.34G>A	p.Gly12Ser	p.G12S
	c.34G>C	p.Gly12Arg	p.G12R
	c.34G>T	p.Gly12Cys	p.G12C
	c.35G>A	p.Gly12Asp	p.G12D
	c.35G>C	p.Gly12Ala	p.G12A
	c.35G>T	p.Gly12Val	p.G12V
	c.[34G>A; 35G>C]	p.Gly12Thr	p.G12T
13	c.37G>T	p.Gly12Cys	p.G13C
	c.38G>A	p.Gly13Asp	p.G13D

Laboratory based methods*2.2.6 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 (31).

2.2.7 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 (32). 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).

2.2.8 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 (33). 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).

2.2.9 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 (34). MALDI-TOF has a limit of detection of approximately 10% tumour DNA in a background of wild-type DNA (35).

2.2.10 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 (36). 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).

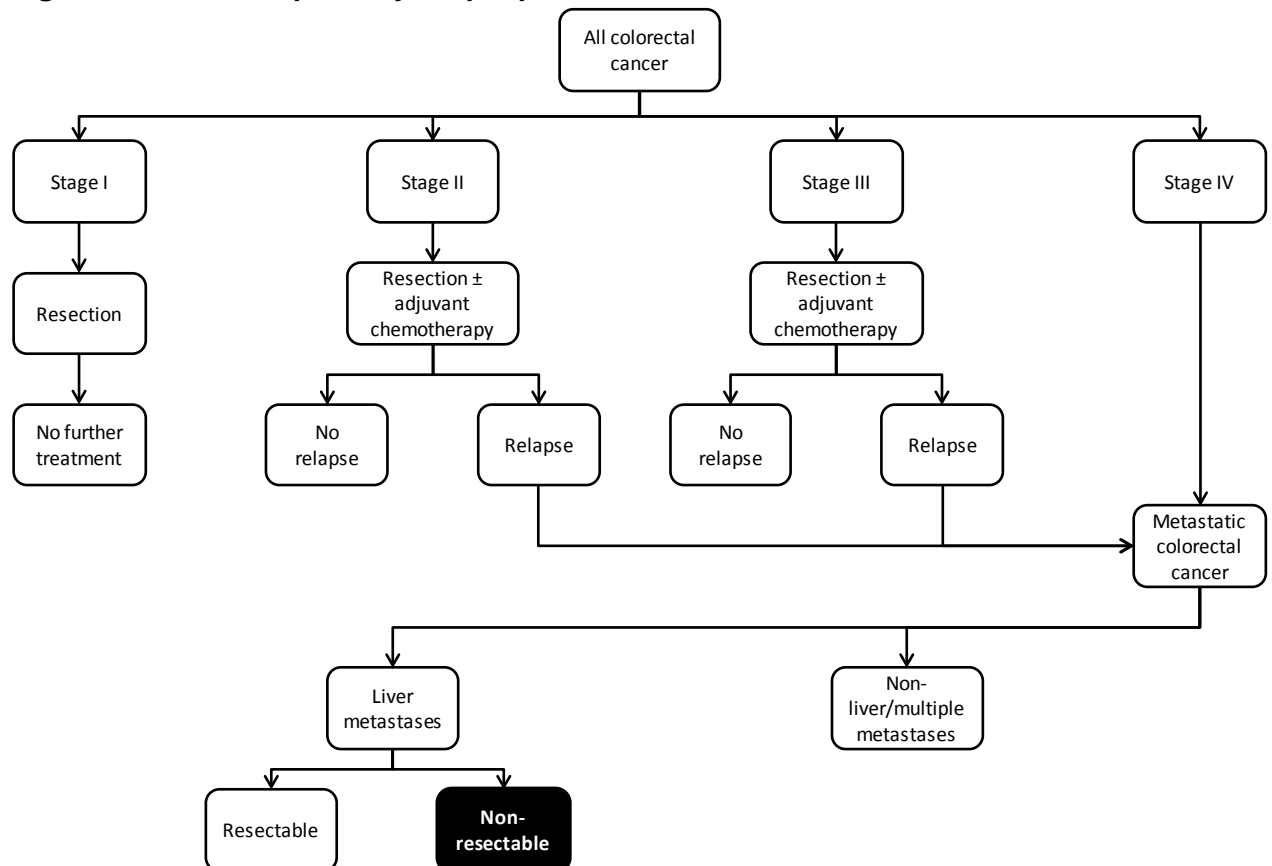
3 Target condition – colorectal cancer

3.1 Background

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 (37). 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) (38). 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% (39).

The population of interest for this evaluation is patients with metastatic colorectal cancer whose metastases are confined to the liver and are unresectable. This group of patients is highlighted in the black box in Figure 1. Of patients with advanced colorectal cancer, between 35% and 40% have mutations in the KRAS oncogene (40).

Figure 1: Treatment pathway for people with colorectal cancer



Adapted from technology appraisal 176 evidence review group report (41)

3.2 Care pathway

3.2.1 Diagnosis

NICE has produced a guideline on [the diagnosis and management of colorectal cancer](#) (2012, NICE clinical guideline 131) (42). This guideline states that for people presenting to secondary care with suspected colorectal cancer, diagnostic investigations would consist of one of the following, dependent upon comorbidities and the specific skills of the local radiology department:

- Colonoscopy
- Flexible sigmoidoscopy then barium enema
- 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 treatment in the second group of patients is therefore 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 (43).

The KRAS status of a tumour is identified through analysis of 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 (44). 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 then referred to if metastatic disease develops. Reflex testing is a more streamlined approach to testing and potentially avoids delays 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 (45;46).

3.2.2 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. However patients with a KRAS mutant tumour would not respond to an epidermal growth factor receptor inhibiting monoclonal antibody, but would experience the toxic side effects. Therefore these patients will gain the most benefit from standard chemotherapy alone. 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 (42).

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](#)) (47).

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 that cetuximab in combination with FOLFOX or FOLFIRI, within its licensed indication, is recommended 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 (48).

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' (49). Therefore patients must have a KRAS mutation test and the tumour be identified as a wild-type KRAS before treatment with cetuximab 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](#). However, these treatments may be given to some patients through the Cancer Drugs Fund. If cetuximab is considered in the third-line setting, the KRAS status of the patient's tumour

is often not retested, but a decision will be made based on the result of the KRAS test performed earlier in the care pathway.

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 (42).

3.3 Patient preferences and issues

Patients have a preference for a minimum number of interventions, for example, they would like to avoid additional biopsies. In terms of the KRAS mutation test, patients have no preference for a specific test, but would like to know that an accurate test is used which can provide results in time to inform treatment decisions. In relation to the potential 2 to 4 week delay associated with testing KRAS tumour status once metastatic disease has developed rather than testing at first diagnosis, this is not a major concern for patients as a first round of standard chemotherapy can be started while waiting for the result of a KRAS mutation test. Once the result is available, cetuximab can be added into the treatment regimen if the tumour is identified as KRAS wild-type.

4 Scope of the evaluation

Table 7: Scope of the evaluation

Decision question	Of the scoped interventions, which technologies / methodologies for KRAS mutation testing in an optimised population of adults with metastatic colorectal cancer are clinically effective and cost-effective for informing first line treatment decisions as currently recommended by NICE in technology appraisal 176, in the NHS in England?
Population	Adults with previously untreated metastatic colorectal cancer in whom:

	<ul style="list-style-type: none"> • 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 cetuximab.
Interventions	<p>Tests for inclusion in the assessment are:</p> <ul style="list-style-type: none"> • 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 of codons 12, 13 and 61 (in-house method) • Pyrosequencing of codons 12, 13 and 61 (in-house method) • MALDI-TOF mass spectrometry on codons 12, 13 and 61 (in-house method) • Next generation sequencing of codons 12, 13 and 61 (in-house method)
Comparator	A range of methods for KRAS mutation testing are currently used in NHS laboratories. Although not a gold standard, Sanger sequencing of codons 12, 13 and 61 will be the comparator for the purpose of the economic modelling.
Healthcare setting	Secondary or tertiary care
Outcomes	<p>Intermediate measures for consideration may include:</p> <ul style="list-style-type: none"> • Number of true positives / false positives / true negatives / false negatives for the prediction of treatment benefit • Minimum % tumour cells in sample needed (limit of detection) • Failure rate • Turnaround time
	<p>Clinical outcomes for consideration may include:</p> <ul style="list-style-type: none"> • Tumour response rate • Resection rates of metastases • Survival (overall and progression free) • Treatment related adverse events

	<ul style="list-style-type: none"> • Health related quality of life
	<p>Costs will be considered from an NHS and Personal Social Services perspective. Costs for consideration may include:</p> <ul style="list-style-type: none"> • Costs for KRAS mutation testing, including an option where Merck Serono meet testing costs • Costs associated with administration of an EGFR inhibiting monoclonal antibody within current NICE recommendations • Costs associated with curative intent liver surgery • Costs associated with ongoing care and support following resection of metastases • Costs associated with second and third line treatment • Costs associated with the management of adverse events associated with treatment
	<p>The cost-effectiveness of interventions should be expressed in terms of incremental cost per quality-adjusted life year.</p>
	<p>The costs and benefits associated with testing of KRAS mutation status at first diagnosis of colorectal cancer (reflex testing) compared to waiting until metastatic disease has developed (demand testing) should be described.</p>
Time horizon	Patient's lifetime

5 Modelling approach

5.1 Existing models

The manufacturer submissions for the NICE single technology appraisal of [Cetuximab for the first-line treatment of metastatic colorectal cancer](#) (technology appraisal guidance 176) included a de novo cost-effectiveness model. This model compares first line chemotherapy regimens, FOLFOX4 and FOLFIRI, on their own and in combination with cetuximab in a population of metastatic colorectal cancer patients that have EGFR expressing, KRAS wild-type tumours. A full description of this model is available in the [manufacturer submission](#) document (50) and the model is critiqued in the [evidence review group report](#) (41).

5.2 Modelling possibilities

5.2.1 Availability of evidence

One end-to-end study of the theascreen KRAS RGQ PCR Kit was identified. This was a retrospective analysis of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) CO.17 phase 3 study of cetuximab plus best supportive care (BSC) versus BSC alone. All patients included in the

study had failed previous chemotherapy regimens for the treatment of metastatic colorectal cancer; therefore the study is not directly relevant to the population included in the scope of this evaluation. The original analysis of KRAS mutation status was performed using Sanger sequencing, however, tumour samples were also retrospectively analysed using the theascreen KRAS RGQ PCR Kit (4). The results supported the clinical utility of the theascreen KRAS RGQ PCR Kit for use to identify patients with metastatic colorectal cancer for treatment with cetuximab. This study was the basis of FDA approval of theascreen KRAS RGQ PCR Kit as a companion diagnostic for cetuximab (51).

Two end-to-end studies of an early version of the KRAS LightMix assay were identified. The CRYSTAL study investigated the efficacy of cetuximab plus FOLFIRI as a first-line treatment for patients with metastatic colorectal cancer. The OPUS study investigated the efficacy of cetuximab plus FOLFOX4 as a first-line treatment for patients with metastatic colorectal cancer. Both studies retrospectively screened patients for KRAS mutations in codons 12 and 13 with the use of a PCR clamping and melting curve method implemented via a LightMix KRAS assay from TIB MolBiol (20;21).

In the COIN study KRAS mutations in codons 12, 13 and 61 were screened with both pyrosequencing and MALDI-TOF mass array (Sequenom). This was a study of cetuximab and standard chemotherapy for the first line treatment of advanced colorectal cancer. More than 1000 samples were successfully analysed by both techniques with greater than 99% genotype call concordance. For discordant genotype calls, Sanger sequencing was used to establish the KRAS mutation status (52).

5.2.2 Use of existing models

The assumptions used in the model submitted by Merck Serono for the appraisal of 'Cetuximab for the first line treatment of metastatic colorectal cancer' (technology appraisal 176) should be used to inform the development of a de novo model. This will ensure consistency between the modelling approaches used in the appraisal of cetuximab (technology appraisal 176) and the assessment of diagnostic methods for KRAS mutation testing. This assessment will not update technology appraisal 176.

5.2.3 Clinical significance of KRAS mutations

The CRYSTAL and OPUS studies of cetuximab analysed subgroups of patients with and without mutations in KRAS codons 12 or 13 of their tumours. It was found that tumours with mutations in these codons have a lack of response to cetuximab. However, of tumours without mutation in KRAS codons 12 and 13, not all respond to cetuximab. Mutations in KRAS codon 61

are also known to confer resistance to cetuximab, and there is some evidence to show that mutations in codon 146 are also linked to a lack of response to anti-EGFR monoclonal antibodies (53). In addition, there are conflicting reports on the clinical significance of mutation G13D.

There is also uncertainty over whether a very sensitive KRAS mutation test (one which identifies low levels of mutant alleles in tumour cells) is always identifying clinically significant mutations. Some evidence suggests that highly sensitive methods for KRAS testing do improve the identification of patients with metastatic colorectal cancer who are resistant to anti-EGFR monoclonal antibodies (35).

Further, approximately 8% of patients who have a KRAS wild-type tumour will have a BRAF V600E mutation which has also been associated with resistance to anti-EGFR monoclonal antibodies. It is not currently routine practice to test BRAF mutation status and it is outside the scope of this evaluation to analyse the effect of mutations other than KRAS mutations.

5.2.4 Funding of KRAS testing

A large proportion of KRAS mutation testing in the UK is currently funded by Merck Serono. The funding is available for all patients with colorectal cancer regardless of whether they have early stage or metastatic disease. Merck Serono has agreements in place with a variety of private sector and NHS laboratories. They do not state a specific test to be used, but do require that the tests are validated and that the laboratories participate in an external quality assurance scheme. Despite this funding, the NHS is still paying for some KRAS mutation testing directly. The modelling should include scenarios where all KRAS mutation testing is funded by Merck Serono, and where all KRAS mutation testing is funded by the NHS.

5.2.5 Costs and benefits of reflex testing

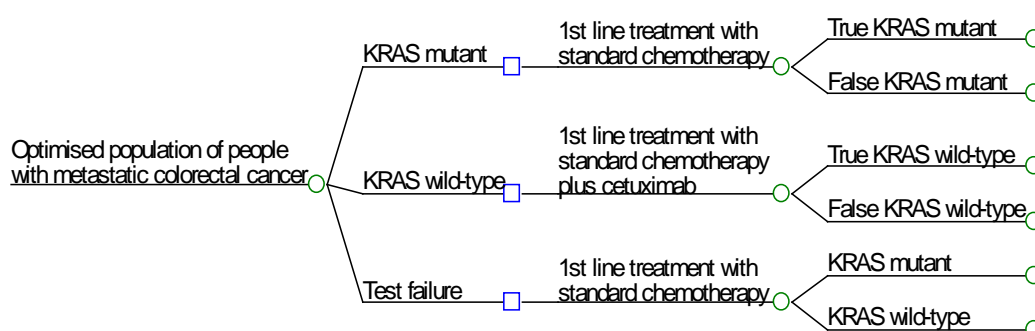
If the KRAS status of patient's tumour is tested at first diagnosis of colorectal cancer, it is likely the overall costs of testing would increase. This would be due to an increased number of test requests compared to if testing were performed when metastatic disease developed. Using a reflex test strategy, at least 20% of tests performed would be redundant as metastatic disease would never develop. However, potential benefits may arise through streamlining of service provision and economies of scale associated with testing. As these costs and benefits may be difficult to quantify, it is suggested that any information collected should be presented in a descriptive format rather than included in a full cost-effectiveness estimate.

5.2.6 Potential model structure

A potential decision tree for modelling is presented in Figure 2. Patients entering the model have a confirmed diagnosis of metastatic colorectal cancer and meet the criteria listed in the NICE recommendation on cetuximab for the first line treatment of metastatic colorectal cancer' (technology appraisal 176) as described in section 3.2.2.

Following a decision on first line treatment, the model should follow patients through the different health states, for example, treatment response, resection of liver metastases, surveillance, second and third line treatments, and death.

Figure 2: Potential decision tree for KRAS mutation testing



6 Equality issues

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.

No other equality issues were identified.

7 Implementation issues

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.

Appendix A Glossary of terms

ARMS

Selective amplification of specific mutation sequences of DNA using Taq DNA polymerase

Codon

A series of three adjacent bases in a single chain of a DNA or RNA molecule, which codes for a specific amino acid

Colon resection

A surgical procedure in which a section of the large intestine is removed

Epidermal growth factor receptor (EGFR)

A cell membrane spanning protein which binds with epidermal growth factor and sends signals which promote growth and differentiation

FOLFIRI

A chemotherapy combination consisting of folinic acid, fluorouracil (5FU) and irinotecan

FOLFOX

A chemotherapy combination consisting of folinic acid, fluorouracil (5FU) and oxaplatin

KRAS gene

The KRAS gene makes the KRAS protein, which is involved in cell signalling pathways, cell growth, and apoptosis (cell death)

Limit of detection

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

Liver resection

The surgical removal of part of the liver

Scorpions

Bi-functional molecules containing a PCR primer covalently linked to a probe for the detection of amplification. The fluorophore in the probe interacts with a quencher, also incorporated into the probe, which reduces fluorescence. During PCR, when the probe binds to the amplified DNA, the fluorophore and quencher become separated. This leads to an increase in fluorescence from the reaction tube.

XELOX

A chemotherapy combination consisting of capecitabine and oxaliplatin

Appendix B Abbreviations

ARMS	Amplification refractory mutation system
CT	Computed tomography
EGFR	Epidermal growth factor receptor
EMQN	European Molecular Genetics Quality Network
FFPE	Formalin fixed and paraffin embedded
HRM	High resolution melt
KRAS	Kirsten rat sarcoma viral oncogene homolog
MALDI-TOF	Matrix assisted laser desorption ionisation-time of flight
NEQAS	National External Quality Assessment Service
NICE	National Institute for Health and Clinical Excellence
PCR	Polymerase chain reaction

Appendix C Related NICE guidance

Cancer service guidance

- Improving outcomes in colorectal cancer. Cancer service guidance (2004). Available from: <http://guidance.nice.org.uk/CSGCC>

Clinical guideline

- Colorectal cancer: the diagnosis and management of colorectal cancer. NICE clinical guideline CG131 (2011). Available from <http://guidance.nice.org.uk/CG131> Date of review: TBC. CG131 updates and replaces [TA93 Irinotecan, oxaliplatin and raltitrexed for advanced colorectal cancer](#), and incorporates [TA100 Capecitabine and oxaliplatin in the adjuvant treatment of stage III \(Dukes' C\) colon cancer](#) and [TA105 Laparoscopic surgery for the treatment of colorectal cancer](#) and [TA61 Capecitabine and tegafur uracil for metastatic colorectal cancer](#)

Technology appraisals

- Colorectal cancer (metastatic) 2nd line: cetuximab, bevacizumab and panitumumab (review). NICE technology appraisal guidance TA242 (2012). Available from: <http://guidance.nice.org.uk/TA242>. Date for review: January 2015. Replaces [TA150 Colorectal cancer \(metastatic\) - cetuximab \(terminated appraisal\)](#) and partially updates [TA118 Colorectal cancer \(metastatic\) - bevacizumab and cetuximab](#)
- Bevacizumab in combination with oxaliplatin and either fluorouracil plus folinic acid or capecitabine for the treatment of metastatic colorectal cancer. NICE technology appraisal guidance TA212 (2010). Available from: <http://guidance.nice.org.uk/TA212>. Date for review: TBC.
- Cetuximab for the first line treatment of metastatic colorectal cancer. NICE technology appraisal guidance TA176 (2009). Available from: <http://guidance.nice.org.uk/TA176>. The last review decision was in June 2011, when it was agreed that TA176 would be cross referenced with CG131. The reason given for not incorporating TA176 into CG131 was "...as the results of the further subgroup analyses of the COIN study could potentially lead to the need to update the recommendations in the future."

NICE pathways

- NICE Pathway (November 2011) Colorectal cancer. Available from: <http://pathways.nice.org.uk/pathways/colorectal-cancer>

Quality standards

- Colorectal cancer. NICE quality standard QS20 (August 2012). Available from: <http://guidance.nice.org.uk/QS20>

Under development

- Aflibercept for the treatment of metastatic colorectal cancer which has progressed following prior oxaliplatin-based chemotherapy. NICE technology appraisal (publication expected October 2013). <http://guidance.nice.org.uk/TA/Wave0/617>

Terminated

- Panitumumab in combination with chemotherapy for the treatment of metastatic colorectal cancer (terminated NICE technology appraisal TA240). “NICE is unable to recommend the use in the NHS of panitumumab in combination with chemotherapy for the treatment of metastatic colorectal cancer because no evidence submission was received from the manufacturer or sponsor of the technology.” (December 2011). <http://guidance.nice.org.uk/TA240>

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Appendix E Equality impact assessment – scoping

The impact on equality has been assessed during this assessment according to the principles of the NICE Equality scheme.

1. <i>Have any potential equality issues been identified during the scoping process (scoping workshop discussion, assessment subgroup discussion), and, if so, what are they?</i>
No

2. <i>What is the preliminary view as to what extent these potential equality issues need addressing by the Committee?</i>
N/A

3. <i>Has any change to the draft scope been agreed to highlight potential equality issues?</i>
No

4. <i>Have any additional stakeholders related to potential equality issues been identified during the scoping process, and, if so, have changes to the stakeholder list been made?</i>
No

Approved by Associate Director (name): ...Nick Crabb.....

Date: 21/12/2012

Appendix F Attendees of the assessment subgroup meeting

The following people were in attendance at the assessment subgroup meeting held on 18th December 2012:

	Name	Job Title	Organisation
Standing Committee Members	Dr Simon Fleming	Consultant in Clinical Biochemistry and Metabolic Medicine	Royal Cornwall Hospital
Specialist Committee Members	Jennie Bell	Head of Cancer Genetics Programme	Birmingham Women's NHS Foundation Trust
	Dr Newton ACS Wong	Consultant Histopathologist	Bristol Royal Infirmary
	Dr Phil Chambers	Genomics Facility Manager	Leeds Institute of Molecular Medicine
	Dr Mark Harrison	Consultant Oncologist	Mount Vernon Cancer Centre
	Kate Lloyd	Lay Representative	-
External Assessment Group	Marie Westwood	Review Manager	Kleijnen Systematic Reviews Ltd
	Penny Whiting	Review Manager	Kleijnen Systematic Reviews Ltd
	Manuela Joore (via teleconference)	Health Economist	Maastricht University Medical Centre
	Thea van Asselt (via teleconference)	Health Economist	Maastricht University Medical Centre
NICE Staff	Professor Adrian Newland	Chair, Diagnostics Advisory Committee	NICE
	Nick Crabb	Associate Director	NICE
	Frances Nixon	Technical Analyst	NICE
	David Lyons	Temporary Administrative Support	NICE
	Jae Long	Administrator	NICE