NICE National Institute for Health and Care Excellence

KRAS mutation testing of tumours in adults with metastatic colorectal cancer

Diagnostics Assessment Report (DAR) - Comments

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Consultee 1: **Roche Diagnostics (Manufacturer)**	**1.**	**11-16**	**Summary**	Based on the evidence considered, we agree with the over-all findings that there was not enough evidence to suggest that any one test would be preferred over the other. However, we would like to highlight some severe limitations of the evidence considered and the resulting analysis: • The exclusion of non-UK studies on technical assay performance resulted in no published data being considered. However, for CE-marked test the technical performance according to CE documentation/studies should be a valid source of this information. • Technical performance was based on a questionnaire only that may have methodological limitations. • Accuracy of prediction of response was only possible for two tests and a comparison of the values is very problematic due to the differences in the respective clinical trials. • KRAS mutation test seem to be predominantly funded by the manufacturer of cetuximab. It is therefore not clear if there are established NHS costs for KRAS mutation testing and if there are differences in costs between test (rather than between laboratories). With a large proportion of test manufacturer funded, testing would incur no NHS costs (i.e. cost of testing is essentially included in the medication costs). • Based on the severe limitations on clinical and costs data, the economic analysis is inconclusive	With respect to studies reporting the technical performance of KRAS mutation tests: Studies of this type were included, for the UK only, with the aim of collecting data similar to that provided by the survey of laboratories (i.e. data on the real practical experience of UK laboratories in processing clinical samples and not technical characteristics of the test as required for CE marking). Studies which reported only technical performance or agreement between two testing methods were not considered relevant to this assessment, as these types of study do not provide data which can be used to link test result to clinical outcome. The limitations of the available data and the problems of comparing the performance of different tests where data are derived from different trials were fully described in the report. The limitations of the economic analyses and the limited availability of data on the 'true' costs of testing were also fully described in the report. In addition, test costs only constitute a very small proportion of the total costs, so differences in true test costs are not expected to have a large impact on relative cost-effectiveness.

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				and does not seem to add value to the decision problem.	
	2.	**22**	**2.2.2**	Table 1 in the DAR, lists a Limit of Detection range for cobas KRAS of 1.6 to 6.3%. We believe that to be an error of transcription from Table 1 in Lee et al. The manuscript includes data demonstrating ≥5% limit of detection.	As indicated in the report, the information in table one was taken from a initial contact with UK laboratories by NICE and from a NEQAS report; no publications were referenced as data were not derived from published articles.
	3.	**37**	**3.2.1.**	The fact that only UK laboratory studies were considered in this appraisal led to no study on technical performance of the test being considered. This is too selective as it cannot be expected that such studies performed for CE-marking and validation are repeated in each country. Manufacturer's documentation should be a valid source of this information for this appraisal. In our opinion, excluding high quality peer-reviewed studies on technical performance of assays, such as Whitehall *et al* (Whitehall V, Tran K, Umapathy A, Grieu F, Hewitt C, Evans TJ, et al. A multicenter blinded study to evaluate KRAS mutation testing methodologies in the clinical setting. J Mol Diagn 2009;11(6):543-552), and considering technical performance on a questionnaire only is a missed opportunity to consider a wider range of evidence for decision making in the absence of sufficient (i.e. any) published UK data.	See response to comment 1 above. The Whitehall study was identified by the systematic review process, but was excluded as it does not include any data which would allow a link to be made between the performance of KRAS mutation tests and clinical outcome. Studies of this type are not informative as they only show agreement between two tests which essentially have different definitions of a positive mutation (different target mutations and limits of detection). If tests results are not related to clinical outcome, then it is not possible to determine whether a mutation detected by one test and not the other would in fact have resulted in more appropriate/effective treatment.

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	4.	**43**	**3.2.1**	Table 5: The technical performance reported in the survey is likely to be influenced by differences in laboratory practices and reporting bias as highlighted in the discussion and the findings by UK-NEQAS (p. 95 of the report). For example, it is not always clear how failure rates were estimated, e.g. whether they were due to technical failures of the assay or other reasons and whether they were 1st pass estimates or based on re-testing of samples where possible. The quality of the reported data also seems to vary between laboratories/tests. We would also like to comment that the failure rate of the cobas KRAS test as reported in the Gonzalez de Castro et al. (Gonzalez de Castro D, Angulo B, Gomez B, Mair D, Martinez R, Suarez-Gauthier A, Shieh F, Velez M, Brophy VH, Lawrence HJ, Lopez-Rios F. A comparison of three methods for detecting KRAS mutations in formalin-fixed colorectal cancer specimens. Br J Cancer. 2012 Jul 10;107(2):345-51) and Harle et al.(Harlé A, Busser B, Rouyer M, Harter V, Genin P, Leroux A, Merlin JL. Comparison of COBAS 4800 KRAS, TaqMan PCR and high resolution melting PCR assays for the detection of KRAS somatic mutations in formalin-fixed paraffin embedded colorectal carcinomas. Virchows Arch. 2013 Mar;462(3):329-35) papers were 1.7 and 3.8%, respectively. Due to high variability in sample quality, invalid rates are difficult to assess without direct comparison between methods on the same specimens. cobas KRAS has well-defined quality metrics to prevent reporting of results for a sample of insufficient quality. It is unclear from the questionnaire whether LDT methods have similar	We acknowledge the limitations of the survey approach. However, given the minimal amount of relevant published data. We feel that this approach represented the best possibility for gaining insight on UK practice experience. The utility, or otherwise, of information collected in this way should be discussed at the DAC. We acknowledge the importance of direct comparisons between tests, made using the same study population and this limitation in the currently available data was noted in the report. However, as noted above, we do not believe that studies such as Gonzalez de Castro et al. and Harle et al. are informative to this assessment. This is because they only show the extent of agreement between two tests which essentially have different definitions of a positive mutation (different target mutations and limits of detection). If tests results are not related to clinical outcome, then it is not possible to determine whether a mutation detected by one test and not the other would in fact have resulted in more appropriate/effective treatment. The two studies cited do provide some information on relative failure rates, but this information was not derived from routine clinical practice in the UK and both studies therefore failed to meet the inclusion

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				quality measures.	criteria for this assessment.
	5.	**44**		Table 6: there seems to be limited reliable cost data on the true opportunity cost of the test to the NHS. This might be due to the low volume of test done or the fact that costing was not carried out by laboratories because the majority of tests are funded by the manufacturer of cetuximab. The cost of the test therefore does not seem to fall directly on the NHS at present.	Comment only – no response needed
	6.	**50**	**3.2.2**	Accuracy values used were not adjusted for differences in definition of response, patient groups and treatment in the different trials. In our opinion it is therefore very problematic to compare the resulting values.	Given the very limited data available, it was not possible to adjust accuracy data to account for other differences between trials. The limitations of the available data and the assumptions made were fully discussed in the report.
		55	**3.2.2**	The scope of the appraisal considered studies comparing cetuximab with standard chemotherapy compared to chemotherapy alone. However, we would like to point out that a recent study of panitumumab may indicate differences in response based on RAS mutations. In the PRIME study, KRAS codon 61 improved prediction of non-response (see amgen press release and http://www.asco.org/sites/www.asco.org/files/abstract_115136.pdf). This may lead to differentiation between KRAS assays that is not evident when considering cetuximab data alone.	The scope of this appraisal was in line with current NICE guidance on the treatment of inoperable metastatic colorectal cancer where metastases are limited to the liver (TA176). Panitumumab is not currently recommended for this application and studies of KRAS mutation testing prior to treatment with panitumumab were therefore not considered relevant to this assessment.
	7.	**81**	**4.3.1**	Resource use costs associated with KRAS mutation testing: we agree that based on the limited data equal test costs should be assumed. However, these might not be the true resource costs to the NHS due to the funding of the majority	Indeed, part of the test costs may at this moment not be included in the actual price that the labs have reported. However, one might reasonably assume that if the manufacturer of cetuximab is

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				of the test by the manufacturer of cetuximab. The analysis would need to indicate/confirm that the assumption is that these were the test costs if the NHS was to fund the test, and £127 may be an underestimate.	now paying for the test, the test costs will be included in the price of cetuximab, which is funded by the NHS. And whether or not the price used in the analysis is an underestimate, it would not impact the relative cost-effectiveness now reported in the results.
	8.	**85**	**4.3.1**	Linked evidence analysis: this analysis is very problematic as it is based on an indirect comparison. Accuracy values used were not adjusted for differences in definition of response, patient groups and treatment in the trials (see comments on 3.2.2.). In our opinion the results are therefore biased to the extent that they do not allow robust conclusions on the relative cost-effectiveness of the tests.	We agree with the stakeholder that the results of this analysis (and all other analyses in this report) should be interpreted with extreme caution, because of the limitations mentioned. However, as mentioned in a previous response, the data did not allow for adjustment of accuracy estimates to account for other differences between trials. Therefore, in our opinion, the linked evidence analysis was the best option available given the limited data.
	9.	**92**	**4.4.2**	We do not consider the results of the sensitivity analysis meaningful based on the assumptions made and the fact that differences in effect and cost modelled are very small compared to the overall costs and effects in the different arms. The result that "cobas was least effective and least costly" is based on questionnaire data only. We think the result may be biased by differences in laboratory practices and quality of the estimate provided in the survey in terms of technical performance.	The sensitivity analysis that the reviewer is referring to is not entirely based on survey results. It is only the technical failure rate and test costs (equal for all tests) which are taken from the survey here. We do however acknowledge that survey results may indeed be biased, which is why we have only used the survey data on technical failures in a sensitivity analysis.
Consultee 2: **Royal College of Pathologists**	**1.**	**24**	**2.3.1**	**'If a sample is stored as FFPE for a long time this can lead to DNA degradation resulting in higher chance of test failure'** Although this is true it does not account for the major	Comment only – no response needed We did not attempt to assess the possible effects of different types of sample processing/storage on test performance (outside the scope of this

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				reason for failure as newer samples also fail, the important factor is the length of time the specimen is in fixative which can vary – often a problem with larger specimens or resections. Also the type of fixative used can be of relevance. The effect is seen as increased cross linking of DNA which can compromise analysis.	assessment). This topic may warrant discussion at the DAC.
	2.	**12**	**Para 1**	The approach of using patient outcomes to assess the effectiveness of a test contains some potential pitfalls. The critical aspect of this type of testing resides with the limits of detection and the ability to produce consistent and reliable results. This is inherently challenging in this particular set of sample types due to tumour heterogeneity, mutation load and sample preparation. Drawing too many conclusions directly to patient outcomes contains too many other influencing factors.	We acknowledge that drawing comparisons between tests, with respect to clinical outcome, is problematic given the limited data currently available. However, as previously noted, a judgement based solely on technical performance (e.g. limit of detection) would not be valid, since it is unclear whether the ability of a test to detect lower levels of mutation will translate into clinical benefit.
Consultee 3: **Royal College of Nursing**	**1.**			There are no comments to submit on behalf of the Royal College of Nursing to inform on the above Diagnostic Assessment Report. Thank you for the opportunity to participate.	No response needed.
Consultee 4: **TIB Molbiol (Manufacturer)**	**1.**	**20**	**2.2.1**	The assay based on the PCR amplification of a 166 bp fragment from exon 2 (first coding exon) and downstream intron of the human K-ras gene covering the codons 12 and 13. The detection of mutations is realized by a subsequent melting curve analysis with hybridization probes, reporting the presence of any mutation in the sample. In order to reduce the amplification of the wild type target	No response needed – the consultee provides technical information about the test characteristics, but no data linking test performance to treatment selection or clinical outcome.

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				the reaction mix contains a wild type specific competitor oligonucleotide, thus enriching all contained mutations, as published for conventional PCR by Thiede et al. (1996). The modified method describing the competition with a detection probe and subsequent melting curve analysis was published 2004. More recently the competitor has been changed to a Locked-Nucleic Acid (LNA) oligomer (Beranek et al., 2007). The competitor oligonucleotide also affects the amplification of the mutated target. Samples with low amounts of target DNA might be inhibited even thought the content of mutated tumor cells is high. Thus a modified cycling protocol was developed comprising a target-enrichment step followed by a mutation-specific PCR. Within the target-enrichment the total DNA should be amplified whereat the presence of the competitor oligonucleotide favours the amplification of the mutated DNA. In contrast to the target-enrichment step the mutation-specific PCR consists of an additional incubation step at 81°C at which the competitor oligonucleotide binds selectively to the wild-type DNA leaving only the mutated DNA as amplification target. This modified PCR cycling protocol results in a significant higher sensitivity of the assay detecting reliable mutations also in samples with low total DNA amount or a with a low content of mutated cells. The TIB Molbiol LightMix Kit K-ras Codons 12/13 consists of two reactions as presented in Figure 1. To ensure a significant amount of amplifiable genomic DNA each sample has to be tested with a Clinical-Sample-Verification-PCR (CSV-PCR). Giving a melting peak the DNA amount in	

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				the sample can be declared as sufficient and thus as a valid sample. Within the Mutant-Specific-Reaction (MSR-PCR) the sample is analyzed in the presence of the competitor oligonucleotide reporting a mutation in case of a melting peak or a K-ras wild-type sample if no melting peak is obtained. The test can be run on the Capillary LightCycler® Instrument LC1.x and LC2.0 as well as on the multiwell plate-based LightCycler® 480 Instrument (Roche).	
	2.	**41**	**3.2.1**	The TIB MOLBIOL LightMix Kit K-ras Codons 12/13 can be performed in less than 3 hours, depending on the used DNA-extraction kit, the number of samples and the used LightCycler Instrument. The TIB MOLBIOL LightMix Kit K-ras Codons 12/13 was evaluated with the High Pure PCR Template Preparation Kit (Roche) for which a total handling time of approximately 30 to 120 minutes can be assumed. Depending on the number of samples a total time for assay-setup of 30 minutes can be estimated. Cycling time strongly depends on the LightCycler Instrument. For the capillary LightCycler Instrument the total cycling time was determined to be 90 minutes while for the plate-based LightCycler 480 Instrument the total cycling time is approximately 180 minutes.	See previous response.

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	3.	**42**	**3.2.1**	The sensitivity of the assay was determined by a dilution series of cloned plasmids containing the mutations p.G12S, p.G12R, p.G12C, p.G12D, p.G12A, p.G12V, p.G12T, p.G13C and p.G13D in a background of 500 ng and 10 ng human placenta DNA representing the most likely range for genomic DNA prepared from FFPE extracts. The human placenta DNA was purchased from was detected to be KRAS-wt. The detection limits were obtained to be less than 0.8 percent for a background of 500 ng and - depending on the mutation- from 0.8 to 6.5 percent in a background of 10 ng wt-DNA.	See previous response.
	4.	**44**	**3.2.1**	The TIB Molbiol LightMix Kit K-ras Codons 12/13 provides premixed primers and probes sufficient for 48 PCR reactions, FastStart DNA polymerase (Roche Diagnostics, Mannheim, Germany), magnesium chlorid and positive controls for the mutations p.G12C and p.G13D. The list-price is €990,00 and allows the analysis of 42 samples on LightCyler 2.0 Instrument and of 46 samples on LightCycler 480 Instrument. Depending on the number of samples tested within the same LightCycler 2.0 Instrument run and including LightCycler-specific consumables (LightCycler capillaries, LightCycler PCR plates) the costs per samples were estimated €24,70 (14 samples) to €64,86 (one sample). The LightCycler 480 Instrument allows analysis of 46 samples in parallel. The costs were estimated to be €21,83 (46 samples) to €66,86 (one sample).	Although this is interesting information, the costs mentioned here do not represent NHS prices and do not include DNA-extraction, personnel, or overhead within the labs, so it does not provide a test cost that could be used in the cost-effectiveness analysis.

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				Above calculation does not include instrument costs as they are consideres as standard Real-Time-PCR instrumentation available in many laboratories. Howeve, using current market price for LightCycler 2.0 and 480 instruments of 40,000 € and estimating a life time of five years and one run per day the costs per run are 40 € which has to be distributed on the costs per sample (minimum 1 € per sample). This cost calculation does not include DNA-extraction, staff costs and standard laboratory consumables.	
	5.	**111**	**6.1**	A high sensitivity of the TIB MOBIOL LightMix Kit K-ras Codons 12/13 was mentioned earlier by Vicki Whitehall et al. (Vicki Whitehall, Kayla Tran, Aarti Umapathy, Fabienne Grieu, Chelsee Hewitt, Tiffany-Jane Evans, Tuty Ismail, Wei Qi Li, Peter Collins, Paul Ravetto, Barbara Leggett, Manuel Salto-Tellez, Richie Soong, Stephen Fox, Rodney J. Scott, Alexander Dobrovic, and Barry Iacopetta (2009) A Multicenter Blinded Study to Evaluate KRAS Mutation Testing Methodologies in the Clinical Setting. Journal of Molecular Diagnostics, 11: 543-551). She reported an unexpected high frequency of muations in FFPE tissue detected with the TIB Molbiol LightMix kit and came to the conclusion that the kit detects false positive sample and is therefore not suitable for FFPE tissue. We have not seen any original data but we assume that this is due to the higher sensitvity compared Sanger sequencing which is the gold standard.	See response to Consultee 1, Comment 3.

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				Weichert et al. has reported that sequencing, analysis with the Chipron array and our LightMix kit gave identical results and comparable numbers of false results. In contrast to Vicki Whitehall's argumentation Mariella Dono et al. also reported a very high sensitivity for the TIB Molbiol LightMix Kit but brought the results in correlation with the clinical follow-up survey of these patients who were detected KRAS-negative with the Therascreen test and received an anti-EGFR treatment. For those cases who were KRAS wild-type detected with both tests a response to the therapy of 37 percent was found, while for these patients who were KRAS-wt (Therascreen) and KRAS mt (TIB Molbiol) the response occurred to be only 18 percent. Similar results were reported by Maria Arcila et al. for using a LNA based technique. The results of these studies indicate that even a few KRAS-mutated cells might have a non-negligible effect on the efficiency of an anti-EGFR therapy and also the necessity of a very high sensitiv KRAS-detection assay.	As noted previously, studies which assessed only agreement between two testing methods (as was the case for both the Arcila and the Weichert studies), without a link to treatment choice or clinical outcome, were not considered relevant to this appraisal. Both of these studies were identified during the review process, but were excluded from this assessment. The study by Mariella Dono et al. appears to have been indexed on MEDLINE after completion of the searches for our systematic review and was therefore not assessed for inclusion. However, this article did not meet the inclusion criteria for our review as: 1. It was not clearly conducted in participants with unresectable CrC metastases confined to the liver. 2. Not all participants received standard chemotherapy as described by TA176.# 3. Data on response to treatment were not sufficiently complete to allow estimates of test accuracy to be derived (as described in Table 2 of our report). As noted by the consultee, this study does provide some initial indication that detection low-level mutations may be clinically relevant. This article may be of interest for discussion at the DAC.
	6.	**114**	**7.**	The following publications were not considered for the present study even though they compare different	See response to comment 5.

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				methodologies for the detection of KRAS mutations: Mariella Dono, Carlotta Massucco, Silvana Chiara, Claudia Sonaglio, Marco Mora, Anna Truini, Giannamaria Cerruti, Gabriele Zoppoli, Alberto Ballestrero, Mauro Truini, Manlio Ferrarini, Simona Zupo (2012) Low percentage of KRAS mutations revealed by LNA-PCR: implications for treatment of mCRC. *Mol. Med.* 18: 1519-1526 Maria Arcila, Christopher Lau, Khedoudja Nafa, Marc Ladany (2011) Detection of KRAS and BRAF Mutations in Colorectal Carcinoma. *J. Mol. Diagnos.* 13: 64-73 Wilko Weichert, Christiane Schewe, Annika Lehmann, Christine Sers, Carsten Denkert, Jan Budczies, Albrecht Stenzinger, Hans Joos, Olfert Landt, Volker Heiser, Christoph Röcken, Manfred Dietel (2010) KRAS Genotyping of Paraffin-Embedded Colorectal Cancer Tissue in Routine Diagnostics. *J. Mol. Diagnos.* 12: 35-42	
Consultee 5 **NHS Professional**	**1.**	**18**	**2.2**	Codon 12 and 13 of the *KRAS* gene are located in exon 2 not exon 1 of the gene. Exon 1 is non-coding (reference sequence NM_033360.2).	This error will be corrected ahead of publication.
	2.	**22**	**2.2.2**	In Table 1 Sanger sequencing limit of detection is stated as unclear. A study of 9 UK labs comparing sensitivity of different methods, which includes Sanger sequencing, for detection of EGFR mutations is in press in the Journal of Diagnostic Molecular Pathology (manuscript attached).	See response to Consultee 1, Comment 2; the information in table 1 is derived directly from UK laboratory experience of KRAS mutation testing.
	3.	**42**	**3.2.1**	In Table 4 the first entry under Sanger sequencing with a tumour load requirement of over 30% states that	The reporting of survey results does not identify specific responding laboratories and, as the

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				microdissection is used. This entry refers to the survey response from our laboratory but we do not perform microdissection.	consultee's affiliation is not given, we cannot fully check this comment. However, checking of the raw survey data shows a response of 'yes' to the question on use of microdissection associated with a laboratory reporting use of Sanger sequencing and having a tumour load requirement of >30%.

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Consultee 6 **NHS Professional**		**12**	**Para 1**	Summary implies inclusion of unknown KRAS result with mutant KRAS results for treatment choices. If this was also done in the data analysis then the analysis will have been compromised by inappropriately including unknown KRAS wildtype patients in the KRAS mutation group, particularly as mutation is less frequent than wild-type.	This paragraph refers to the assumptions on treatment pathways which were used in the economic modelling only and not to any results reported in the systematic review.
		12	**Para 1**	The approach of using patient outcomes to assess the effectiveness of a test is flawed. The test should be judged on its ability to detect the presence or absence of a specific biological marker. Using patient outcomes explores the utility of the biomarker rather than the method used to detect it. There is little difference in the effectiveness of different tests (kits or in-house), either through the content of each test (which mutations are included) or in each tests performance (detection sensitivity etc). However, there are major differences in outcomes dependent on treatment choices (there are several standard chemotherapy options) and other clinical parameters (including mutation profiles that have not been tested for). Thus any potential difference in test performance (small) will be masked by the large differences in outcomes due to other factors. The significant assumptions linking test results to outcomes reinforces this problem. Assessing the cost-effectiveness of knowing the KRAS mutation status through health economic modelling is clearly important – and should drive the decision of whether to test or not. However, linking this to the method of detection is not likely to be helpful (see next comment). Perhaps based on current methods NICE should be	As noted in previous responses, we acknowledge the limitations of the available data. However, we do not believe that a comparison of tests on technical performance characteristics only represents a valid approach. This is because different test methodologies may detect different combinations and levels of mutations and comparison of technical performance alone does not allow a link to be made between test result by a given method and clinical outcome. We agree with the comment that the current available evidence does not allow robust conclusions to be drawn on relative cost-effectiveness of any test. However, since we cannot rule out a relationship between test method and clinical outcome, as argued above, there may also be a difference in cost-effectiveness between tests, independent of technical performance and test costs alone.

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				recommending minimum performance characteristics and maximum costs for suitable tests – laboratories would then continue to be responsible for choice of test (which would take into account their available technologies) and the effectiveness of their strategy assured through EQA (which could be strengthened by central infrastructure support).	
		15	**Para 1**	This paragraph observes that clinical effectiveness of the therapy was not determined by which test strategy was used. This outcome was entirely predictable.	Comment only – no response needed
		15	**Research Priorities**	Could also include looking at cost effectiveness of combine testing of KRAS with other gene mutations, e.g. gene mutations panels by Next Generation Sequencing.	This was outside the scope of the current assessment.
		15	**Research Priorities**	Raises potential of quantitative assays in longitudinal studies. Could also include assessment of significance of level of KRAS mutation at initial testing for therapeutic decision making, i.e. to explore the significance of the mutation when it is only present initially in a minor subclone. This is rightly discussed on page 106, section 5.3.1. para 1; but does not make it into Research Priorities.	Detailed research recommendations should be discussed at the DAC. With this in mind, we have delayed our response to the NIHR request for prioritised research recommendations until after the DAC.
		15	**Research Priorities**	Could also include assessment of significance of less common KRAS mutation, particularly those in codons 61 and 146. This is rightly discussed on page 106, section 5.3.1. para 2; but does not make it into Research Priorities. Such research could be a model for other tumours. There is evidence of differential significance of mutations in other oncology fields.	See previous response
		21	**Para 2**	Considering Next Generation Sequencing is important and	NGS may require particular consideration under

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		Also page 189		inclusion of this in this paragraph is welcomed. However, comparing NGS to Sanger sequencing leaves the impression that it has the same limitations regarding 25% tumour DNA as detection limit, whereas NGS will work with much lower concentrations of tumour DNA. Within this context it is also important to recognise the potential of NGS for multiplexing with other gene mutation targets, and to provide quantitative data.	recommendations for research.
		112	**6.1 End of last para**	NGS for KRAS and other mutations (including EGFR and BRAF for example) via a panel test is now in routine use in the CR-UK Stratified Medicine Programme. TSB Stratified Medicine Innovation Platform funded projects are also now producing NGS panels (See Oxford Gene Technology and LifeTech) or other panel profiling tools (see Affymetrix Oncoscan). Although NGS was not in use at the time of the survey it is important to acknowledge that the field has already moved on considerably.	See previous response
		113	**Para 1**	NGS will also provide quantitative results, and also relative quantitation with respect to other mutations in the same panel.	Comment only – no response needed

*The comments of Lab21 were moved from the Model Feedback form to this form as these comments were not considered specific comments on the economic model rather comments on the estimation of the test results and test costs. The presumed page numbers were filled out by the AEG.