National Institute for Health and Care Excellence

Final

Melanoma: assessment and management

[A] Evidence reviews for genetic testing for melanoma

NICE guideline NG14

Evidence reviews underpinning recommendations 1.3.8 to 1.3.14 and research recommendations in the NICE guideline

July 2022

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1 Genetic testing for melanoma

1.1 Review question

What is the role and optimal timing of genetic testing of the tumour after diagnosis for a person with melanoma?

1.1.1 Introduction

The BRAF gene plays a role in the regulation of cellular growth, and mutations of the BRAF gene can cause uncontrolled cell growth. There is uncertainty as to the role of immunohistochemistry (IHC), a rapid form of genetic testing, in the diagnosis of V600e BRAF mutations in people with stage IIC and III melanoma. The diagnostic accuracy of IHC, compared to gold standard tests such as next generation sequencing (NGS) and COBAS 4800 will be evaluated in this review, irrespective of when the genetic testing was conducted.

Input from topic experts during the 2019 surveillance review of NG14 highlighted there was a need to updates recommendations on genetic testing in view of the increased availability of effective adjuvant therapies and the introduction of the 8th edition of the American Joint Committee on Cancer staging system and the 8th edition of the Union for International Cancer Control (UICC) Tumour Node Metastasis (TNM) staging system for melanoma.

This review is part of an update of the NICE guideline on melanoma: assessment and management (NG14, 2015). This guideline covers adults and children with melanoma. This guideline will also cover all settings in which NHS care is received or commissioned.

1.1.2 Summary of the protocol

Table 1 PICO table for genetic testing for people with melanoma

Population	People with a diagnosis of stage I-IV melanoma
Index test	• BRAF immunohistochemistry testing
Reference standard	• COBAS 4800 (with or without confirmation of discordant cases) • Next-generation sequencing (as defined by study) Data will be separated by reference standard.
Outcomes	• Sensitivity/specificity • Likelihood ratios Data will be separated into diagnostic accuracy for detecting V600e mutations and for detecting any V600 mutations

1.1.3 Methods and process

This evidence review was developed using the methods and process described in Developing NICE guidelines: the manual. Methods specific to this review question are described in the review protocol in appendix A and the methods document.

Declarations of interest were recorded according to NICE's conflicts of interest policy.

The original review question focused on people with stage IIC-III melanoma. However, the population was expanded to include all stages due to limited evidence specific to stage IIC/III and because diagnostic accuracy should not be affected by disease stage.

Diagnostic accuracy data is reported for two mutation outcomes: the presence of specifically V600e mutations and the presence of any V600 mutation.

For the detection of V600e mutations, a positive IHC result is deemed to be a true positive when the reference standard detects specifically a V600e mutation, with any other mutation resulting in a false positive. A negative IHC result is deemed to be a true negative when the reference standard detects wild-type mutation or a non-V600e mutation, with a false negative only possible when the reference standard detects a V600e mutation.

For the detection of all V600 mutations, a positive IHC result is deemed to be a true positive when the reference standard detects any BRAF mutation, with a false positive occurring when the reference detects a wild-type mutation (or a non-V600 mutation however these are typically not explored in studies). A negative IHC result is deemed to be a true negative when the reference standard detects a wild-type mutation or non-V600 mutation, a false negative occurs when the reference standards detect any V600 mutation.

1.1.4 Diagnostic accuracy evidence

1.1.4.1 Included studies

A systematic literature search was conducted for this review on genetic testing for people with melanoma. This returned 5,231 references (see appendix B for the literature search strategy). Based on title and abstract screening against the review protocol, 5,150 references were excluded, and 81 references were ordered for screening based on their full texts.

Of the 81 references screened as full texts, 3 references met the inclusion criteria specified in the review protocol for this question (appendix A) and were specific to people with stage 2C-3 melanoma. Following discussion with the committee it was agreed that the inclusion criteria should be expanded to all people with melanoma (regardless of stage), increasing the final number of references to 13. The clinical evidence study selection is presented as a diagram in appendix C.

Re-run searches identified an additional 1 reference for inclusion.

1.1.4.2 Excluded studies

See Appendix I for a list of references for excluded studies, with reasons for exclusion.

1.1.5 Summary of studies included in the effectiveness /diagnostic/prognostic evidence review

Table 2 Summary of included studies characteristics

Author (year)	Country	Sample size	Population	Reference standard	Risk of bias	Directness
Barel (2018)	France	36 samples	Advanced melanoma	NGS	Low	Directly applicable
Ronchi (2021)	Italy	50 samples	Melanoma	NGS	Low	Directly applicable
Ehsani (2014)	USA	25 samples	Metastatic malignant melanoma	COBAS	Moderate	Directly applicable

Author (year)	Country	Sample size	Population	Reference standard	Risk of bias	Directness
Fisher (2014)	USA	118 sample	Malignant melanoma	COBAS Discordant cases confirmed with NGS	Moderate	Directly applicable
Franczak (2017)	France	59 samples	Melanoma	• COBAS • NGS	Low	Directly applicable
Ihle (2014)	Germany	63 samples	Melanoma	• COBAS • NGS	Low	Directly applicable
Lade-Keller (2013)	Denmark	28 samples	Melanoma	COBAS confirmed using pyrosequencing or sanger	Moderate	Directly applicable
Lo (2016)	UK	152 samples	Melanoma	COBAS	Moderate	Directly applicable
Nielsen (2018)	Denmark	224 samples	Metastatic melanoma	COBAS (V2) Confirmed using Quiagen	Low	Directly applicable
O'Brien (2017)	Ireland	112 samples	Metastatic melanoma	COBAS	High	Directly applicable
Schirosi (2016)	Italy	64 samples	Metastatic melanoma	COBAS	High	Directly applicable
Sener (2017)	Turkey	98 samples	Metastatic melanoma	COBAS (V2) confirmed using pyrosequencing	Moderate	Directly applicable
Tetzlaff (2015)	USA	154 samples	Melanoma	NGS	Moderate	Directly applicable
Uguen (2015)	France	104 samples	Melanoma	Pyrosequencing confirmed using NGS	High	Directly applicable

See appendix D for full evidence tables.

1.1.6 Summary of the diagnostic evidence

Table 3 Summary of GRADE tables assessing accuracy of IHC

Reference standard	No. studies (sample size)	Diagnostic accuracy			Quality
		Sensitivity	Specificity	Likelihood ratios	
COBAS 4800					
Main analysis	9 (837)	0.90 (0.86, 0.93)	0.92 (0.81, 0.97)	LR+ 12.45 (4.62, 33.49)	Very low
				LR- 0.11 (0.08, 0.15)	Moderate
Sensitivity analysis *excluding high risk of bias studies*	7 (686)	0.91 (0.86, 0.94)	0.91 (0.76, 0.97)	LR+ 10.41 (3.58, 30.32)	Very low
				LR- 0.11 (0.07, 0.15)	Moderate
COBAS 4800 (discordant cases between COBAS and IHC confirmed using third testing method)					
Main analysis	6	0.89	0.98	LR+ 55.49 (24.23,127.06)	Moderate

Reference standard	No. studies (sample size)	Diagnostic accuracy			Quality
		Sensitivity	Specificity	Likelihood ratios	
	(745)	(0.82, 0.94)	(0.96, 0.99)	LR- 0.11 (0.06, 0.19)	Low
Next-generation sequencing					
Main analysis	5 (393)	0.80 (0.65, 0.90)	0.98 (0.93, 0.99)	LR+ 28.10 (10.15, 77.79)	Low
				LR- 0.22 (0.12, 0.40)	Very low
Sensitivity analysis *excluding high risk of bias studies*	4 (289)	0.83 (0.63,0.93)	0.97 (0.91, 0.99)	LR+ 22.92 (7.67, 68.49)	Moderate
				LR- 0.26 (0.18, 0.38)	Very low
Assessing only v600e	5 (383)	0.95 (0.87,0.98)	0.96 (0.92, 0.98)	LR+ 25.46 (12.35, 52.49)	Moderate
				LR- 0.06 (0.02, 0.14)	Moderate
Assessing only v600e *excluding high risk of bias studies*	4 (279)	0.94 (0.85,0.98)	0.96 (0.91, 0.98)	LR+ 22.25 (10.52, 47.09)	Moderate
				LR- 0.06 (0.02, 0.17)	Moderate

See appendix F for full GRADE tables.

1.1.7 Published economic evidence

1.1.7.1 Included studies

A single search was performed to identify published economic evaluations of relevance to any of the questions in this guideline update (see appendix B). This search retrieved 7,545 studies. Based on title and abstract screening, 7,538 of the studies could confidently be excluded for this question. 7 studies were excluded following the full-text review. Thus, the review for this question does not include any study from the existing literature.

1.1.7.2 Excluded studies

See Appendix I for excluded studies and reasons for exclusion.

1.1.8 Economic model

The committee prioritised this question for original modelling. Table 4 provides a brief summary of methods and results.

1.1.9 Summary of Economic evidence

Table 4: Summary of economic evidence

Study	Applicability	Limitations	Incremental			Uncertainty
			Cost (£)	Effects	ICER (£/Effect)	
De novo model (2021) Cobas vs. IHC & Cobas	Directly applicable	Potentially serious limitations	For Stage IIC Costs: Cobas: £75,179	For Stage IIC Effects:	For Stage IIC Incremental: Costs: £53,554	**Stage IIC:** **Deterministic:** Most sensitive to the cost of IHC testing.

Study	Applicability	Limitations	Incremental Cost (£)	Incremental Effects	Incremental ICER (£/Effect)	Uncertainty
			IHC & Cobas: £128,751 For Stage III Costs: Cobas: £75,179 IHC & Cobas: £128,751	Cobas: 67.14 IHC & Cobas: 76.06 For Stage III Effects: Cobas: 195.22 IHC & Cobas: 221.21	Effects: 9.91 For Stage III Incremental: Costs: £53,554 Effects: 26.01	**Probabilistic:** Congruent to deterministic results. Stage III: **Deterministic:** Most sensitive to the cost of IHC testing. **Probabilistic:** Congruent to deterministic results.

1.1.10 The committee's discussion and interpretation of the evidence

1.1.10.1. The outcomes that matter most

The committee agreed that that both sensitivity/specificity and likelihood ratios were suitable methods of visualising the diagnostic accuracy data and that the quality assessment should be done on the likelihood ratios due to the existence of an established interpretation of these values, making it easier to assess imprecision.

IHC has the benefit of being conducted very quickly compared to standard tests for BRAF mutations, such as COBAS, and there is the potential for IHC to be used to detect BRAF mutations when immediate treatment is required and an urgent test is required, such as for people with fast progressing disease. IHC is deemed to produce very few false positive results and therefore a positive test could be used to diagnose BRAF mutation without further testing. As IHC only detects V600e mutations, a negative test would always require further testing to determine BRAF mutation status. Based on this, the committee agreed that specificity and positive likelihood ratios were the most important outcomes when assessing immunohistochemistry.

The committee agreed that it was appropriate to assess the accuracy of IHC for detecting all BRAF mutations and for detecting specifically v600e mutations. The former approach would reflect the accuracy of IHC when used in practice, and the latter would reflect the accuracy of IHC for what it was designed to do as IHC only aims to detect v600e mutations.

A false positive would result in a person being incorrectly staged and being classified as BRAF mutant. This may lead to them receiving targeted treatment instead of a more suitable therapy, such as adjuvant pembrolizumab or other immunotherapies. False positive patients will not respond to targeted treatment in the expected manner as they do not possess the BRAF mutation, and will eventually need to switch to another treatment, potentially after experiencing disease progression.

A false negative would not have significant downstream consequences. The person would go on to receive the previous standard of care – BRAF analysis with a COBAS test – to confirm or exclude BRAF mutation.

A true positive result would result in the person being correctly upstaged, classified as BRAF mutant and becoming eligible for additional treatment options.

A true negative result would result in the person being correctly classified as BRAF wild-type and their staging would be unaffected.

1.1.10.2 The quality of the evidence

All evidence came from retrospective cohort studies that were directly applicable to the review question. Studies were typically of low risk of bias. Areas in which there was a risk of bias stemmed from a lack of blinding or the use of composite reference standards, in which a person's true BRAF status was determined by one of numerous possible tests, allowing different samples to undergo different reference standards.

The committee advised that in clinical practice IHC would be used to rule-in people with a BRAF mutation, with a positive result classifying someone as BRAF-positive and a negative result requiring that the person undergo subsequent testing with COBAS or an alternative genomic BRAF test.

Studies reported a variety of different reference standards. The committee advised that it was important to look at studies using COBAS as the reference standard (either COBAS alone, or COBAS with subsequent testing for discrepant cases between COBAS and IHC) as in practice, decisions about subsequent treatment are based on the results of the COBAS test alone.

However, the committee agreed that as COBAS also has the potential for false negative and false positive results, there is a risk that this would lead to an inaccurate measure of the diagnostic accuracy of IHC and that the reference standard of NGS is preferable as this is a true gold standard test that would allow a comparison between IHC and the person's actual mutation status. It was agreed that the economic model would primarily use data where diagnostic accuracy was assessed using NGS as a reference standard. They agreed that studies using COBAS as a reference standard was still useful as it would give an indication of what would happen when the tests are used sequentially.

When using COBAS alone as a reference standard, there was a high degree of heterogeneity between studies in their reported positive likelihood ratios. Heterogeneity was still present when using NGS as the reference standard although it was less pronounced.

1.1.10.3 Benefits and harms

The committee advised that evidence suggests that people with clinical stages IIA-C have similar 5- and 10-year mortality rates, comparable to those with stage IIIA-B melanoma. People with stage IIC are at a particularly high risk of mortality, with evidence suggesting 5- and 10- year mortality rates slightly higher than those with stage IIIB melanoma. The committee highlighted challenges with retrieval of genetic samples from storage and that it was more practical to test for BRAF status at the point of diagnosis rather than when they would become eligible for targeted therapy, e.g. upon progression, as delays to treatment due to sample retrieval and testing time can cause harms to patients who may be at risk of further deterioration. The committee also wished to recommend that BRAF analysis of melanoma tissue samples be arranged by the local MDT in order to provide a more coordinated process. The pathology report on the primary lesion should also include the relevant tissue block suitable for molecular genetic testing, as determined by the dermatopathologist within the MDT.

The committee agreed that BRAF testing is essential to identify whether people are eligible for targeted therapies and, although recommendations made in evidence review F deprioritise use of these treatments in people with unresectable stage III or IV, there is still a significant portion of people who would receive targeted therapy as first line treatment if they

were at risk of rapid progression, preferred to use targeted therapy after consideration of the safety profile compared with immunotherapy agents, or who would switch to targeted therapies after immunotherapy. Additionally, targeted therapies are used in adjuvant settings for people with resectable stage III melanoma.

The committee also agreed that although people with stage IIA-C disease would not immediately benefit from having their BRAF status known, testing these people at the point of diagnosis has practical utility. A significant portion of people with stage IIA-C disease will relapse and having their BRAF status already known will speed up decisions surrounding which treatment to give.

Based on this evidence the committee agreed to recommend that BRAF testing be offered to people with clinical stage IIC-IV melanoma and be considered for people with clinical stage IIA or IIB melanoma.

The committee agreed to keep recommendations that BRAF analysis not be used in people with stage IA-IB melanoma due to the low risk of BRAF mutation and better prognosis in these groups of people.

The committee agreed that false positive results with IHC are very rare and that the diagnostic accuracy evidence confirms this, due to the high specificity. As IHC only detected the V600e BRAF mutation, many people with a (non-V600e) BRAF mutation will be missed if they were to be tested by IHC alone. This is reflected in the evidence for the sensitivity of IHC, which is smaller in comparison to its specificity. Although V600e is the most common form of BRAF mutation in people with melanoma, other variants (particularly V600k) are also common. Economic modelling estimated that IHC followed by PCR Cobas is more expensive than PCR Cobas alone, but results in a greater number of people appropriately receiving targeted therapy.

The committee agreed that the faster speed in which IHC can be processed (in hours instead of days/ weeks) is a clear clinical benefit to IHC. This is particularly important in people with poorer prognosis (such as people with metastatic cancer) who require rapid treatment. They also agreed that after a sample is taken, there are difficulties establishing the stage of disease.

As such, the committee agreed that IHC be considered as the first test for samples undergoing BRAF analysis but that negative tests should go on to receive confirmatory testing using an alternative BRAF genomic test.

The committee were aware that availability of the necessary equipment and technical expertise to analyse and interpret IHC assays will vary between centres and that particularly for smaller centres who only administer a small number of BRAF tests, it may not be cost effective or feasible for them to purchase the necessary equipment. They accounted for this possibility in the recommendations. Additionally, the committee agreed that decisions to test for BRAF mutations should take into account suitability for targeted or systemic therapy if they were to test positive.

1.1.10.4 Cost effectiveness and resource use

No published economic evidence was identified from the systematic review. However, the committee was presented with economic evidence from a *de novo* cost consequence analysis developed for the guideline. The model assessed the costs and effectives of different approaches to genetic testing at diagnosis for identifying BRAF mutations in patients with stage IIC and III melanoma. The testing approaches compared were PCR Cobas alone versus using upfront immunohistochemistry (IHC) with PCR Cobas reserved for only those patients who test negative with IHC.

In advising on an appropriate structure for the model, the committee noted that the greatest benefit of genetic testing with IHC is the reduced test turnaround time compared to PCR Cobas (e.g., same day result vs a waiting period of 14 days), which avoids delays in patients receiving adjuvant targeted therapies or systemic targeted therapies on recurrence. However, IHC is limited in its ability to detect all BRAF mutations and can only identify patients with BRAF *V600E* mutations. This means that if one were to test with only IHC, a number of patients with other actionable BRAF mutations (e.g., BRAF *V600K, V600R, V600D, V600M)* would be incorrectly identified as BRAF wildtype. Thus, anyone who tests negative with IHC should then be tested using a secondary genetic test, such as PCR Cobas, which can identify all relevant and actionable BRAF mutations. To capture the negative consequences of a longer test turnaround time, the model was structured so that anyone who receives a PCR Cobas test can either get a test result or die before receiving a test result. The probability of death during the longer test turnaround time although small, potentially has an impact on the number of patients that can ultimately go on to receive targeted therapy. Additionally, the committee was interested in the costs of each testing approach as well as the outcome of the number of patients who go on to appropriately receive targeted therapy as a result of being correctly identified as having a BRAF mutation when using each testing approach.

The committee was presented with the base case model results for two distinct populations, patients with stage IIC melanoma and patients with stage III melanoma. Two different models were built for these two distinct populations as the current treatment pathway for stage IIC is different than stage III melanoma. Currently, those with stage IIC melanoma are only eligible for adjuvant therapy on recurrence, however many of those with resectable stage III melanoma are immediately eligible for adjuvant targeted therapy at diagnosis. The committee also noted that clinical trials are currently ongoing in which could change the pathway of care such that those with stage IIC melanoma would eventually also become eligible for adjuvant therapy immediately at diagnosis rather than only on recurrence. In both patient populations, IHC followed by PCR Cobas is more expensive than PCR Cobas alone, but results in a greater number of people appropriately receiving targeted therapy.

The committee was also presented the results of several deterministic sensitivity analyses, in which the results of the analysis remained largely robust to a range of scenarios when varying any of the model's input parameters within the range of their uncertainty. One parameter that had the greatest effect on the results was the cost of IHC. Probabilistic sensitivity analysis provided congruent results to the base case analysis. The outputs of the probabilistic analysis also provided further support of the model results as there were no iterations for either of the patient population in which the testing approach using IHC with PCR Cobas was associated with worse outcomes compared to PCR Cobas alone.

The committee therefore discussed the appropriateness of the costings used in the model, which relied on data from two micro-costing studies, and agreed that the base case cost of IHC used in the model was likely too low. The micro-costing for IHC in the model was in part based on an IHC micro-costing paper for detection of another mutation. Although the process for IHC would be the same, and therefore our estimates of staff time would be comparable, different antibodies would be needed for BRAF testing. The committee noted the antibody needed for BRAF testing is likely to cost thousands of pounds, which was underestimated in the model at only a few hundred pounds. Individual committee members with knowledge of IHC labs indicated that the cost per IHC test to detect a BRAF V600E mutation may range from £40-£200. However, the higher estimate of £200 was estimated based on validation costs that would be required to set up the IHC platform and would only be incurred within the first year of implementing the test. Therefore, the committee felt that the actual ongoing cost per IHC test would likely to be less than £200. Considering the results of the threshold analyses, the committee felt confident that a testing approach using IHC with PCR Cobas compared to PCR Cobas alone would be highly likely to be cost-effective in patients with stage III melanoma. For patients with stage IIC melanoma, the committee acknowledged that

if the actual cost per test of IHC was £200, which was very close to the upper value of £204 identified in the threshold analysis that a testing approach using IHC with PCR Cobas might not be cost-effective. However, as previously noted, the £200 figure for an IHC test included costs associated with validation, that if they were to be spread over the lifetime of the testing equipment (rather than solely allocated within the first year of testing) the cost per IHC test would be smaller and likely be in the range for the testing approach using IHC with PCR Cobas to be considered cost-effective. Additionally, the committee noted that several labs already have IHC testing capacity and therefore validation of the IHC test for the BRAF V600E mutation might not be as costly and the cost per IHC testing more likely to be in the range to be considered cost-effective (i.e., less than £204). Finally, the committee made note of the fact that one of the primary reasons for the high cost of BRAF V600E mutation testing with IHC is due to one of the antibodies required for the test being on patent. The committee noted that this antibody is due to come off patent in the next few years, which will likely further reduce the cost of testing with IHC increasing the cost-effectiveness of the testing approach.

In view of these considerations, the committee made a recommendation to consider IHC to be used as an initial screening test to identify BRAF V600E mutations alongside a secondary genetic test in those testing negative in patients with either stage IIC or III melanoma.

Although the scope of this review question was limited to those with stage IIC and III melanoma, the committee also made consider recommendations using the same testing approach in patients with stage IIA and IIB melanoma. This was justified based on survival data from the updated AJCC 2018 staging criteria (Gershenwald et al. 2017), where these two populations had similar survival to that of the stage IIC (and IIIA) patients and therefore likely to have similar rates of recurrence. The committee believed that testing may have value in these patients as it would allow them rapid access to therapy upon progression, and that a delay in treatment at this point would be associated with harm if they were rapidly progressing. However, given the uncertainty in making this extrapolation the committee felt it would be better for the recommendations to only consider an IHC with PCR Cobas testing approach, thereby giving the clinician the option to pursue such testing if they thought it would be of use to the patient.

The committee also felt it was important not to mandate the use of IHC with PCR Cobas, as they worried this would result in labs without this testing capacity sending IHC tests elsewhere, which would both increase the costs of this testing approach and the test turnaround time, thereby negating one of the greatest benefits of this testing approach. Therefore, the recommendation indicated that IHC could be used when available, but if it was not available, another genetic test could be used alone.

The committee also considered the potential resource impact of these recommendations. For centres that already have IHC equipment available and already using it as a testing method, the committee noted the recommendations would have only have a small impact on resource use as such centres would only need to purchase the appropriate antibodies for the IHC BRAF test. For centres that do not have IHC equipment, the committee noted that this would increase resource use, both in the form of the upfront costs required to set up IHC testing, validation of the testing approach in the first year, and ensuring staff are trained such that they are skilled enough to appropriately interpret the test. However, on balance, the committee felt that this increase in resource use was not likely to be prohibitively expensive, would be limited to the first year of implementation, and would likely decrease in the future as the required IHC antibodies come off patent. While the committee expressed some uncertainty over the cost, in light of the results of the economic model and their clinical knowledge, the committee agreed these costs were likely to remain small and come with a number of beneficial outcomes including a quicker time to result for those testing positive with IHC and a greater number of people appropriately receiving targeted therapy.

1.1.11 Recommendations supported by this evidence review

This evidence review supports recommendations 1.3.8 to 1.3.14 and the research recommendations on the use of biomarkers in people with melanoma.

1.1.12 References – included studies

1.1.12.1 Effectiveness

Barel, Fanny, Guibourg, Briac, Lambros, Laetitia et al. (2018) Evaluation of a Rapid, Fully Automated Platform for Detection of BRAF and NRAS Mutations in Melanoma. Acta dermato-venereologica 98(1): 44-49

Ehsani, Laleh, Cohen, Cynthia, Fisher, Kevin E et al. (2014) BRAF mutations in metastatic malignant melanoma: comparison of molecular analysis and immunohistochemical expression. Applied immunohistochemistry & molecular morphology: AIMM 22(9): 648-51

Fisher, Kevin E, Cohen, Cynthia, Siddiqui, Momin T et al. (2014) Accurate detection of BRAF p.V600E mutations in challenging melanoma specimens requires stringent immunohistochemistry scoring criteria or sensitive molecular assays. Human pathology 45(11): 2281-93

Franczak, Claire, Salleron, Julia, Dubois, Cindy et al. (2017) Comparison of Five Different Assays for the Detection of BRAF Mutations in Formalin-Fixed Paraffin Embedded Tissues of Patients with Metastatic Melanoma. Molecular diagnosis & therapy 21(2): 209-216

Lade-Keller J, Kristensen LS, Riber-Hansen R et al. (2013) A role for immunohistochemical detection of BRAF V600E prior to BRAF-inhibitor treatment of malignant melanoma?. Journal of clinical pathology 66(8): 723-725

Lo, Michelle Chin I, Paterson, Anna, Maraka, Jane et al. (2016) A UK feasibility and validation study of the VE1 monoclonal antibody immunohistochemistry stain for BRAF-V600E mutations in metastatic melanoma. British journal of cancer 115(2): 223-7

Nielsen, Line B, Dabrosin, Nina, Sloth, Karen et al. (2018) Concordance in BRAF V600E status over time in malignant melanoma and corresponding metastases. Histopathology 72(5): 814-825

O'Brien, Odharnaith, Lyons, Tomas, Murphy, Sandra et al. (2017) BRAF V600 mutation detection in melanoma: a comparison of two laboratory testing methods. Journal of clinical pathology 70(11): 935-940

Schirosi, Laura, Strippoli, Sabino, Gaudio, Francesca et al. (2016) Is immunohistochemistry of BRAF V600E useful as a screening tool and during progression disease of melanoma patients?. BMC cancer 16(1): 905

Sener, Ebru, Yildirim, Pinar, Tan, Ayca et al. (2017) Investigation of BRAF mutation analysis with different technical platforms in metastatic melanoma. Pathology, research and practice 213(5): 522-530

Tetzlaff, Michael T, Pattanaprichakul, Penvadee, Wargo, Jennifer et al. (2015) Utility of BRAF V600E Immunohistochemistry Expression Pattern as a Surrogate of BRAF Mutation Status in 154 Patients with Advanced Melanoma. Human pathology 46(8): 1101-10

Uguen, Arnaud, Gueguen, Paul, Legoupil, Delphine et al. (2015) Dual NRASQ61R and BRAFV600E mutation-specific immunohistochemistry completes molecular screening in melanoma samples in a routine practice. Human pathology 46(11): 1582-91

1.1.12.2 Economic

No economic studies were included for this review question.

Appendices

Appendix A – Review protocols

Review protocol for the role and optimal timing of genetic testing of the tumour after diagnosis for a person with melanoma

ID	Field	Content
0.	PROSPERO registration number	TBC
1.	Review title	Genetic testing (somatic tumour DNA testing) for melanoma
2.	Review question	What is the role and optimal timing of genetic testing of the tumour after diagnosis for a person with stage 2C-3 melanoma?
3.	Objective	Determine the role and optimal timing of genetic testing of the tumour after diagnosis of melanoma
4.	Searches	TBC
5.	Condition or domain being studied	Melanoma
6.	Population	People with a diagnosis of melanoma* *The original review question focused on people with stage IIC-III melanoma. However, the population was expanded to include all stages due to limited evidence specific to stage IIC/III and because diagnostic accuracy should not be affected by disease stage.

7.	Index tests	• Immunohistochemistry testing for BRAF
8.	Comparator/reference standard	• Genetic testing for BRAF using COBAS 4800 PCR test • Genetic testing for BRAF using next-generation sequencing Studies in which discordant cases between COBAS and IHC undergo confirmatory testing using a third method will be kept separate from those studies which do not use confirmatory testing.
9.	Types of study to be included	• Test and treat RCTs • Diagnostic accuracy studies
10.	Other exclusion criteria	• People with skin tumours and cancers other than cutaneous melanoma. This includes ocular melanoma and melanoma arising from mucosal sites. • Non-English language papers
11.	Context	This review is part of an update of the NICE guideline on melanoma: assessment and management (NG14, 2105). This guideline covers adults and children with melanoma. Input from topic experts during the 2019 surveillance review of NG14 highlighted there was a need to updates recommendations on genetic testing in view of the increased availability of effective adjuvant therapies and the introduction of the 8th edition of the American Joint Committee on Cancer staging system and the 8th edition of the Union for International Cancer Control (UICC) Tumour Node Metastasis (TNM) staging system for melanoma. This guideline will also cover all settings in which NHS care is received or commissioned.

12.	Primary outcomes (critical outcomes)	Diagnostic accuracy studies • Sensitivity/specificity • Likelihood ratios • Prevalence of genetic mutation • Test turnaround times
13.	Secondary outcomes (important outcomes)	• Adverse events
14.	Data extraction (selection and coding)	All references identified by the searches and from other sources will be uploaded into EPPI reviewer and de-duplicated. 10% of the abstracts will be reviewed by two reviewers, with any disagreements resolved by discussion or, if necessary, a third independent reviewer. The full text of potentially eligible studies will be retrieved and will be assessed in line with the criteria outlined above. A standardised form will be used to extract data from studies (see Developing NICE guidelines: the manual section 6.4). Study investigators may be contacted for missing data where time and resources allow. Data will be extracted from the included studies for assessment of study quality and evidence synthesis. Extracted information will include: study setting; study population and participant demographics and baseline characteristics; details of the intervention and control conditions; study methodology; recruitment and study completion rates; outcomes and times of measurement and information for assessment of the risk of bias.

15.	Risk of bias (quality) assessment	Risk of bias will be assessed using the appropriate checklist as described in Developing NICE guidelines: the manual.
16.	Strategy for data synthesis	Meta-analyses of outcome data will be conducted for all comparators that are reported by more than one study, with reference to the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al. 2011). Fixed- and random-effects models (der Simonian and Laird) will be fitted for all comparators, with the presented analysis dependent on the degree of heterogeneity in the assembled evidence. Fixed-effects models will be the preferred choice to report, but in situations where the assumption of a shared mean for fixed-effects model is clearly not met, even after appropriate pre-specified subgroup analyses is conducted, random-effects results are presented. Fixed-effects models are deemed to be inappropriate if one or both of the following conditions was met: • Significant between study heterogeneity in methodology, population, intervention or comparator was identified by the reviewer in advance of data analysis. • The presence of significant statistical heterogeneity in the meta-analysis, defined as $I^2 \geq 50\%$. Meta-analyses will be performed in Cochrane Review Manager V5.3
17.	Analysis of sub-groups	Where data are available, analyses will be conducted by Breslow thickness and melanoma stage at the time of diagnosis. **Subgroups (to be investigated irrespective of presence of statistical heterogeneity):** • Pregnant women. • People with a compromised immune system.

		• Children/adolescents
18.	Type and method of review	☒
		☐
		☐
		☐
		☐
		☐
		☐
19.	Language	English
20.	Country	England
21.	Anticipated or actual start date	10/08/2020
22.	Anticipated completion date	01/04/2022
23.	Stage of review at time of this submission	Review stage
		Preliminary searches
		Piloting of the study selection process

		Formal screening of search results against eligibility criteria
		Data extraction
		Risk of bias (quality) assessment
		Data analysis
24.	Named contact	5a. Named contact Guideline updates team 5b Named contact e-mail skincancer@nice.nhs.uk 5e Organisational affiliation of the review National Institute for Health and Care Excellence (NICE) and
25.	Review team members	From the Guideline Updates Team • Caroline Mulvihill • Thomas Jarratt • Brett Doble • Steph Armstrong • Jeremy Dietz • Jemma Deane
26.	Funding sources/sponsor	This systematic review is being completed by the Guideline Updates Team which receives funding from NICE.
27.	Conflicts of interest	All guideline committee members and anyone who has direct input into NICE guidelines (including the evidence review team and expert witnesses) must declare any potential conflicts of interest in line with NICE's code of practice for declaring and dealing with conflicts

		of interest. Any relevant interests, or changes to interests, will also be declared publicly at the start of each guideline committee meeting. Before each meeting, any potential conflicts of interest will be considered by the guideline committee Chair and a senior member of the development team. Any decisions to exclude a person from all or part of a meeting will be documented. Any changes to a member's declaration of interests will be recorded in the minutes of the meeting. Declarations of interests will be published with the final guideline.
28.	Collaborators	Development of this systematic review will be overseen by an advisory committee who will use the review to inform the development of evidence-based recommendations in line with section 3 of Developing NICE guidelines: the manual. Members of the guideline committee are available on the NICE website: https://www.nice.org.uk/guidance/indevelopment/gid-ng10155
29.	Other registration details	None
30.	Reference/URL for published protocol	None
31.	Dissemination plans	NICE may use a range of different methods to raise awareness of the guideline. These include standard approaches such as: • notifying registered stakeholders of publication • publicising the guideline through NICE's newsletter and alerts • issuing a press release or briefing as appropriate, posting news articles on the NICE website, using social media channels, and publicising the guideline within NICE.
32.	Keywords	• Genetic testing • Immunohistochemistry

		• BRAF • Melanoma • Skin cancer • Skin tumour
33.	Details of existing review of same topic by same authors	Update of question 2.5 in NICE Guideline NG14 Melanoma: assessment and management
34.	Current review status	☒
		☐
		☐
		☐
		☐
35..	Additional information	None
36.	Details of final publication	www.nice.org.uk

Appendix B – Literature search strategies

Searches were run on 12th August 2020 in Medline, Medline in Process, Medline epub, the Cochrane Database of Systematic Reviews (CRD/CENTRAL) and DARE (Wiley platform). These searches are presented below

Table 5 Search strategy for Medline

Database: Medline
1 exp Melanoma/ (95112)
2 Skin Neoplasms/ (121069)
3 (melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*).tw. (103532)
4 ((skin or derm* or cutaneous* or epitheli* or epiderm*) adj1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*)).tw. (61495)
5 ((maligna* or melano*) adj2 (freckle* or lesion* or mole* or nev* or naev*)).tw. (24915)
6 (hutchinson* adj2 (freckle* or melano*)).tw. (69)
7 dubreuilh*.tw. (72)
8 (maligna* adj2 lentigo*).tw. (1065)
9 LMM.tw. (868)
10 or/1-9 (251058)
11 Genetic Testing/ (38160)
12 Molecular Diagnostic Techniques/ (11164)
13 DNA Mutational Analysis/ (60177)
14 exp *Immunohistochemistry/ (39074)
15 In Situ Hybridization, Fluorescence/ (42639)
16 (fluorescence* adj2 (hybridisation* or hybridization*)).tw. (602)
17 ((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) adj2 (analys* or test* or techni*)).tw. (249959)
18 ((braf* or b raf or v600* or dna) adj4 mutat* adj4 (analys* or test* or techni*)).tw. (2722)
19 or/11-18 (401966)
20 Proto-Oncogene Proteins B-raf/ (8767)
21 (braf* or b raf or v600*).tw. (12772)
22 or/20-21 (13843)

Database: Medline	
23	Mutation/ (428586)
24	(gene* adj2 (alter* or chang* or modif*)).tw. (113586)
25	(mutation* or mutated or mutating).tw. (603697)
26	or/23-25 (870250)
27	(sensitiv: or predictive value:).mp. or accurac:.tw. (1884066)
28	22 and 26 and 27 (1798)
29	19 or 28 (402913)
30	10 and 29 (8313)
31	limit 30 to english language (7890)
32	animals/ not humans/ (4691424)
33	31 not 32 (7389)
34	limit 33 to (letter or historical article or comment or editorial or news or case reports) (1280)
35	33 not 34 (6109)
36	limit 35 to ed=20131009-20200812 (2235)

Table 5 Search strategy for Medline in progress

Database: Medline in Process	
1	exp Melanoma/ (0)
2	Skin Neoplasms/ (0)
3	(melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*).tw. (11299)
4	((skin or derm* or cutaneous* or epitheli* or epiderm*) adj1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*)).tw. (6308)
5	((maligna* or melano*) adj2 (freckle* or lesion* or mole* or nev* or naev*)).tw. (3104)
6	(hutchinson* adj2 (freckle* or melano*)).tw. (0)
7	dubreuilh*.tw. (0)
8	(maligna* adj2 lentigo*).tw. (72)
9	LMM.tw. (178)
10	or/1-9 (18742)

Database: Medline in Process
11 Genetic Testing/ (0)
12 Molecular Diagnostic Techniques/ (0)
13 DNA Mutational Analysis/ (0)
14 exp *Immunohistochemistry/ (0)
15 In Situ Hybridization, Fluorescence/ (0)
16 (fluorescence* adj2 (hybridisation* or hybridization*)).tw. (41)
17 ((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) adj2 (analys* or test* or techni*)).tw. (34282)
18 ((braf* or b raf or v600* or dna) adj4 mutat* adj4 (analys* or test* or techni*)).tw. (307)
19 or/11-18 (34546)
20 Proto-Oncogene Proteins B-raf/ (0)
21 (braf* or b raf or v600*).tw. (2715)
22 or/20-21 (2715)
23 Mutation/ (1)
24 (gene* adj2 (alter* or chang* or modif*)).tw. (15043)
25 (mutation* or mutated or mutating).tw. (60257)
26 or/23-25 (72686)
27 (sensitiv: or predictive value:).mp. or accurac:.tw. (241005)
28 22 and 26 and 27 (282)
29 19 or 28 (34745)
30 10 and 29 (580)
31 limit 30 to english language (575)
32 animals/ not humans/ (1)
33 31 not 32 (575)
34 limit 33 to (letter or historical article or comment or editorial or news or case reports) (70)
35 33 not 34 (505)
36 limit 35 to dt=20131009-20200812 (432)

Table 6 Search strategy for Medline Epub

Database: Medline Epub
1 exp Melanoma/ (0)
2 Skin Neoplasms/ (0)
3 (melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*).tw. (2130)
4 ((skin or derm* or cutaneous* or epitheli* or epiderm*) adj1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*)).tw. (1115)
5 ((maligna* or melano*) adj2 (freckle* or lesion* or mole* or nev* or naev*)).tw. (428)
6 (hutchinson* adj2 (freckle* or melano*)).tw. (2)
7 dubreuilh*.tw. (0)
8 (maligna* adj2 lentigo*).tw. (23)
9 LMM.tw. (34)
10 or/1-9 (3312)
11 Genetic Testing/ (0)
12 Molecular Diagnostic Techniques/ (0)
13 DNA Mutational Analysis/ (0)
14 exp *Immunohistochemistry/ (0)
15 In Situ Hybridization, Fluorescence/ (0)
16 (fluorescence* adj2 (hybridisation* or hybridization*)).tw. (8)
17 ((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) adj2 (analys* or test* or techni*)).tw. (5019)
18 ((braf* or b raf or v600* or dna) adj4 mutat* adj4 (analys* or test* or techni*)).tw. (47)
19 or/11-18 (5055)
20 Proto-Oncogene Proteins B-raf/ (0)
21 (braf* or b raf or v600*).tw. (468)
22 or/20-21 (468)
23 Mutation/ (0)
24 (gene* adj2 (alter* or chang* or modif*)).tw. (1924)
25 (mutation* or mutated or mutating).tw. (8575)
26 or/23-25 (10179)
27 (sensitiv: or predictive value:).mp. or accurac:.tw. (28410)

Database: Medline Epub
28 22 and 26 and 27 (44)
29 19 or 28 (5085)
30 10 and 29 (127)
31 limit 30 to english language (125)
32 animals/ not humans/ (0)
33 31 not 32 (125)
34 limit 33 to (letter or historical article or comment or editorial or news or case reports) (5)
35 33 not 34 (120)

Table 7 Search strategy for Embase

Database: Embase
1 exp melanoma skin cancer/ or melanoma/ or cutaneous melanoma/ or metastatic melanoma/ or superficial spreading melanoma/ or skin carcinoma/ (154581)
2 skin tumor/ or skin cancer/ or epithelium tumor/ (66239)
3 (melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*).tw. (160636)
4 ((skin or derm* or cutaneous* or epitheli* or epiderm*) adj1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*)).tw. (91839)
5 ((maligna* or melano*) adj2 (freckle* or lesion* or mole* or nev* or naev*)).tw. (39084)
6 (hutchinson* adj2 (freckle* or melano*)).tw. (80)
7 dubreuilh*.tw. (73)
8 (maligna* adj2 lentigo*).tw. (1657)
9 LMM.tw. (1475)
10 or/1-9 (326255)
11 genetic screening/ (86353)
12 molecular diagnosis/ (19331)
13 exp mutational analysis/ (51028)
14 *immunohistochemistry/ (24640)
15 fluorescence in situ hybridization/ (70357)
16 (fluorescence* adj2 (hybridisation* or hybridization*)).tw. (797)

Database: Embase
17 ((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) adj2 (analys* or test* or techni*)).tw. (377913)
18 ((braf* or b raf or v600* or dna) adj4 mutat* adj4 (analys* or test* or techni*)).tw. (5208)
19 or/11-18 (563290)
20 B Raf kinase/ (23166)
21 (braf* or b raf or v600*).tw. (31813)
22 or/20-21 (37247)
23 mutation/ (238308)
24 (gene* adj2 (alter* or chang* or modif*)).tw. (172958)
25 (mutation* or mutated or mutating).tw. (897591)
26 or/23-25 (1080197)
27 (sensitiv: or predictive value:).mp. or accurac:.tw. (2581394)
28 22 and 26 and 27 (4874)
29 19 or 28 (566272)
30 10 and 29 (14254)
31 limit 30 to english language (13768)
32 nonhuman/ not human/ (4652904)
33 31 not 32 (12994)
34 (conference abstract or conference paper or conference proceeding or "conference review" or letter or editorial).pt. (6401316)
35 33 not 34 (8548)
36 limit 35 to dc=20131009-20200812 (3925)

Table 8 Search strategy for Cochrane Wiley

Database: Cochrane Wiley (CRD/CENTRAL)		
D	Search	Hits
#1	MeSH descriptor: [Melanoma] explode all trees	1784
#2	MeSH descriptor: [Skin Neoplasms] this term only	1543

Database: Cochrane Wiley (CRD/CENTRAL)
#3 ((melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*)):ti,ab,kw 5276
#4 (((skin or derm* or cutaneous* or epitheli* or epiderm*) NEAR/1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*))):ti,ab,kw 3904
#5 (((maligna* or melano*) NEAR/2 (freckle* or lesion* or mole* or nev* or naev*))):ti,ab,kw 667
#6 ((hutchinson* NEAR/2 (freckle* or melano*))):ti,ab,kw 9
#7 (dubreuilh*):ti,ab,kw 0
#8 (maligna* NEAR/2 lentigo*) 50
#9 (LMM):ti,ab,kw 111
#10 {or #1-#9} 8319
#11 MeSH descriptor: [Genetic Testing] this term only 384
#12 MeSH descriptor: [Molecular Diagnostic Techniques] this term only 36
#13 MeSH descriptor: [DNA Mutational Analysis] this term only 225
#14 MeSH descriptor: [Immunohistochemistry] explode all trees 4317
#15 MeSH descriptor: [In Situ Hybridization, Fluorescence] this term only 211
#16 ((fluorescence* NEAR/2 (hybridisation* or hybridization*))):ti,ab,kw 225
#17 (((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) NEAR/2 (analys* or test* or techni*))):ti,ab,kw 5133
#18 (((braf* or b raf or v600* or dna) NEAR/4 mutat* NEAR/4 (analys* or test* or techni*))):ti,ab,kw 354
#19 {or #11-#18} 9420
#20 MeSH descriptor: [Proto-Oncogene Proteins B-raf] this term only 154
#21 ((braf* or b raf or v600*)):ti,ab,kw 1238
#22 {or #20-#21} 1238
#23 MeSH descriptor: [Mutation] this term only 1285
#24 ((gene* NEAR/2 (alter* or chang* or modif*))):ti,ab,kw 2522
#25 ((mutation* or mutated or mutating)):ti,ab,kw 12563
#26 {or #23-#25} 14822
#27 #22 AND #26 879

Database: Cochrane Wiley (CRD/CENTRAL)		
#28	#19 OR #27	10156
#29	#10 AND #28	609

Table 9 Search strategy for CRD (DARE)

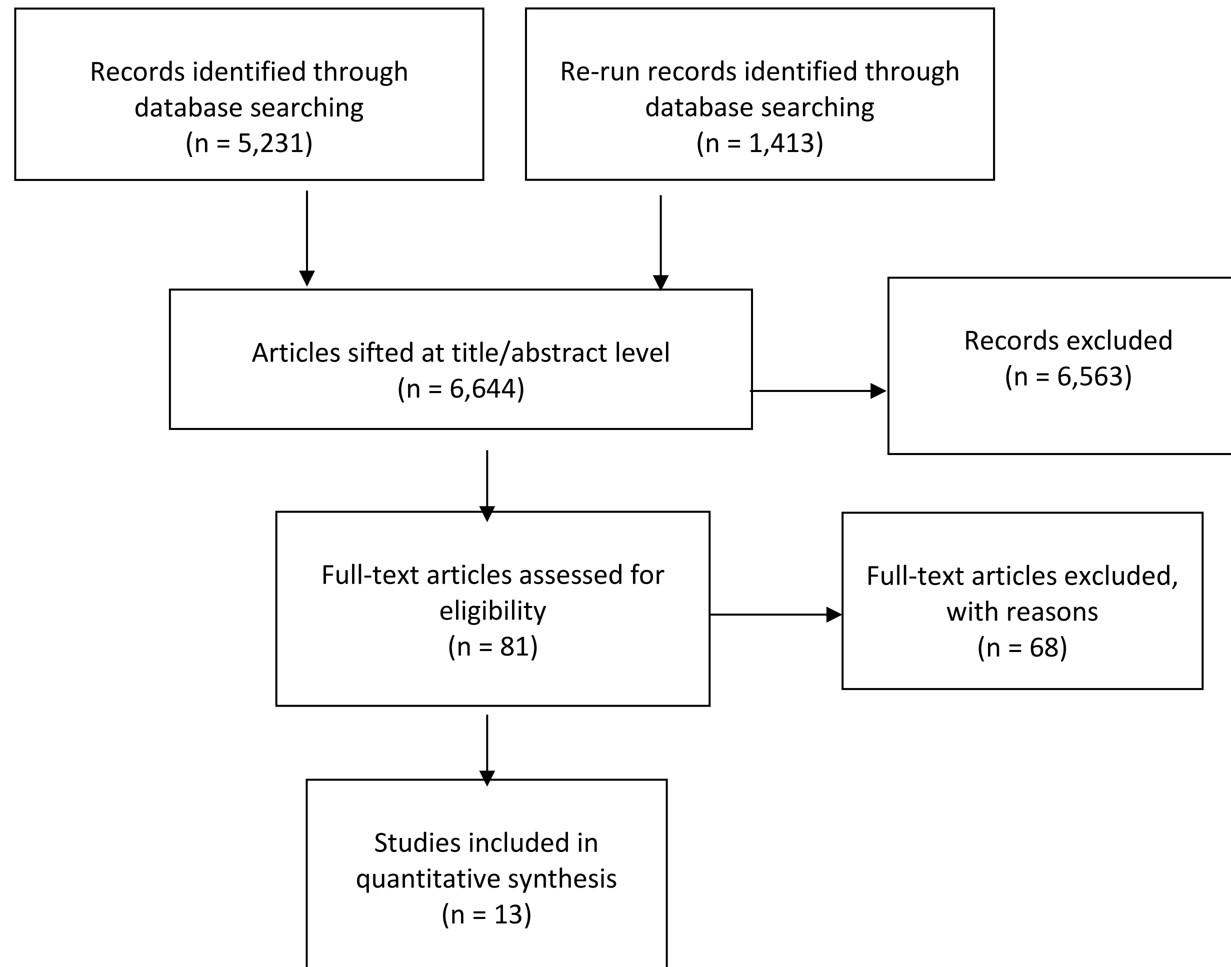
Database: CRD (DARE)

1	MeSH DESCRIPTOR melanoma EXPLODE ALL TREES IN DARE	81
2	MeSH DESCRIPTOR Skin Neoplasms IN DARE	94
3	((melanoma* or melanocarcinoma* or naevocarcinoma* or nevocarcinoma*)) IN DARE	140
4	(((skin or derm* or cutaneous* or epitheli* or epiderm*) NEAR1 (adenocarcinoma* or cancer* or carcinoma* or malignan* or neoplas* or oncolog* or tumor* or tumour*))) IN DARE	203
5	(((maligna* or melano*) NEAR2 (freckle* or lesion* or mole* or nev* or naev*))) IN DARE	65
6	((hutchinson* NEAR2 (freckle* or melano*))) IN DARE	0
7	(dubreuilh*) IN DARE	0
8	((maligna* NEAR2 lentigo*)) IN DARE	0
9	(LMM) IN DARE	0
10	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9	313
11	MeSH DESCRIPTOR Genetic Testing IN DARE	34

Database: CRD (DARE)		
12	MeSH DESCRIPTOR Molecular Diagnostic Techniques IN DARE	22
13	MeSH DESCRIPTOR DNA Mutational Analysis IN DARE	14
14	MeSH DESCRIPTOR Immunohistochemistry EXPLODE ALL TREES IN DARE	131
15	MeSH DESCRIPTOR In Situ Hybridization, Fluorescence IN DARE	10
16	((fluorescence* NEAR2 (hybridisation* or hybridization*))) IN DARE	9
17	(((genetic* or genomic* or immunohistochem* or "immuno histochem*" or IHC or FISH or fluorescen* or immunofluorescen* or "immuno fluorescen*" or molecular* or somatic*) NEAR2 (analys* or test* or techni*))) IN DARE	169
18	(((braf* or b raf or v600* or dna) NEAR4 mutat* NEAR4 (analys* or test* or techni*))) IN DARE	14
19	#11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18	301
20	MeSH DESCRIPTOR Proto-Oncogene Proteins B-raf IN DARE	4
21	((braf* or b raf or v600*)) IN DARE	4
22	#20 OR #21	4
23	MeSH DESCRIPTOR Mutation IN DARE	92
24	((gene* NEAR2 (alter* or chang* or modif*))) IN DARE	27
25	((mutation* or mutated or mutating)) IN DARE	182

Database: CRD (DARE)			
26	#23 OR #24 OR #25	209	
27	#22 AND #26	4	
28	#19 OR #27	305	
29	#10 AND #28	4	

Appendix C – Diagnostic accuracy evidence study selection



Appendix D – Diagnostic accuracy evidence

Barel, 2018

Bibliographic Reference	Barel, Fanny; Guibourg, Briac; Lambros, Laetitia; Le Flahec, Glen; Marcorelles, Pascale; Uguen, Arnaud; Evaluation of a Rapid, Fully Automated Platform for Detection of BRAF and NRAS Mutations in Melanoma.; Acta dermato-venereologica; 2018; vol. 98 (no. 1); 44-49

Study Characteristics

Study type	Diagnostic accuracy study
Study details	Study location France Study dates 2015-2016 Sources of funding none reported
Inclusion criteria	Advanced stage melanoma advanced stages of melanoma according the recommendations of the French National Cancer Institute
Number of participants	36 samples
Index test(s)	Immunohistochemistry BRAF V600E (clone VE1, Spring Bioscience) were used at a dilution of 1:100. IHC was performed on Ventana Benchmark XT® automated slide preparation system, using ultraView Universal Alkaline Phosphatase Red Detection Kit (Roche Diagnostics), as reported previously. UltraView® Red detection kit was used through Ventana staining procedure that included pre-treatment with cell conditioner 1 (pH 8) for 60 min, followed by incubation with diluted antibody at 37°C for 32 min. Antibody incubation was followed by standard signal amplification with the Ventana amplifier kit and ultra-Wash. Slides were counterstained with 1 drop of haematoxylin for 12 min and 1 drop of bluing reagent for 4 min. Immunostaining was interpreted by a single pathologist without knowledge of the molecular status. Staining was considered positive when it was cytoplasmic and moderate to strong, clearly different from the background. It was considered negative when no or only faint or nuclear labelling was noted.
Reference standard (s)	Next generation sequencing Suite software v4.4.0 was used for signal processing, run quality report and Fastq files generation. BRAF and NRAS sequences were then analysed through the SeqNext software v4.1.2 (JSI Medical Systems GmbH, Ettenheim, Germany). Nucleotide numbering was carried out in accordance with Human Genome Variation Society (HGVS) recommendations

	(www.hgvs.org/ mutnomen). The reference sequences NM_004333.4 for BRAF gene and NM_002524.4 for NRAS gene were used for cDNAbased numbering, i.e. the A of the ATG translational initiation codon was ascribed as +1.
Outcome measures	Sensitivity and specificity
Outcome data	Detection of V600E BRAF mutation TN:27 TP:9 FN:0 FP:0 Detection of all V600 mutations TN:21 TP:9 FN:6 FP:0

Study-level characteristics

	Study (N = 36)
Female	44.4%
Primary melanomas	33.3%
Metastatic melanomas *(%)*	66.6%

Risk of bias

Section	**Question**	**Answer**
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low

Section	Question	Answer
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Low**
	Directness	**Directly applicable**

Ronchi, 2021

Bibliographic Reference Ronchi, A., Montella, M., Zito Marino, F., Caraglia, M., Grimaldi, A., Argenziano, G., ... & Cozzolino, I. (2021). Predictive Evaluation on Cytological Sample of Metastatic Melanoma: The Role of BRAF Immunocytochemistry in the Molecular Era. *Diagnostics*, *11*(6), 1110

Study Characteristics

Study type	Diagnostic accuracy study
Study details	Study location Italy Study dates January 2017 and December 2020 Sources of funding none
Inclusion criteria	Diagnosis of CM metastases rendered on FNA samples The realization of a cell-block with presence of residual biomaterial

	Molecular evaluation of BRAF mutational status performed on the same cytological sample or the corresponding histological sample, when surgery was performed.
Number of participants	50 samples (from 50 participants)
Index test(s)	Immunohistochemistry BRAF V600E (clone VE1, Spring Bioscience) were used at a dilution of 1:100. IHC was performed on Ventana Benchmark XT® automated slide preparation system, using ultraView Universal Alkaline Phosphatase Red Detection Kit (Roche Diagnostics), as reported previously. UltraView® Red detection kit was used through Ventana staining procedure that included pre-treatment with cell conditioner 1 (pH 8) for 60 min, followed by incubation with diluted antibody at 37°C for 32 min. Antibody incubation was followed by standard signal amplification with the Ventana amplifier kit and ultra-Wash. Slides were counterstained with 1 drop of haematoxylin for 12 min and 1 drop of bluing reagent for 4 min. Immunostaining was interpreted by a single pathologist without knowledge of the molecular status. Staining was considered positive when it was cytoplasmic and moderate to strong, clearly different from the background. It was considered negative when no or only faint or nuclear labelling was noted. ICC was performed on 4 μm-thick FFPE cell-block slices using a fully automatized assay based on the Ventana® BRAF V600E (VE1, Ventana-Roche Diagnostics, Meylan, France) mouse monoclonal primary antibody in combination with the Ventana OptiView DAB IHC Detection Kit® on the Ventana® Benchmark XT platform (Ventana-Roche Diagnostics, Meylan, France). The procedure was performed according to the manufacturer’s instructions. 2.3. BRAF Immunocytochemistry Evaluation All immunostained slides were evaluated by two cytopathologists in absence of any information about molecular data. Immunostaining was primarily interpreted as positive or negative. We defined a case as positive if it showed diffuse cytoplasmic staining, according to data reported in histological series [13,23]. We considered a case as negative if no staining or only nuclear dot staining was present. Furthermore, the percentage of positive neoplastic cells and intensity of the staining were recorded. The percentage of positive neoplastic cells were calculated by comparing the stained neoplastic cells to the total number of neoplastic cells in the slide
Reference standard (s)	Next generation sequencing The evaluation of the mutational status of the BRAF gene was performed by the NGS method. DNA was extracted from 4 unstained 10 μM FFPE tissue sections or from the cytological samples. DNA was obtained using the QIAamp® DNA FFPE kit Tissue (Qiagen, Hilden, Germany) for histological samples or using the Qiagen QIAamp® DNA Micro kit. (Qiagen, Hilden, Germany) for cytological samples, according to the manufacturer’s instructions. The massive parallel sequencing of DNA libraries by ION Torrent Personal was used as previously reported [7]. Sequencing was carried out using different chips on the Ion Personal Genome Machine System (PGM™, Thermo Fisher Scientific, Waltham, MA, USA). Torrent Suite Software v.4.0.2 (Life Technologies, Carlsbad, CA, USA) to assess run performance and data analysis was used. Integrative Genomics Viewer (IGV v 2.2, Broad Institute, Cambridge, MA, USA) was used for visual inspection of the aligned reads. Data were analyzed using Ion Reporter software [22] and further filtered through quality checking. We selected all SNVs in the studied genes resulting in a non-synonymous amino acid change, or a premature stop codon, and all short indels resulting in either a frameshift or insertion/deletion of amino acids. All SNVs were analyzed for previously reported hotspot mutations (somatic mutations reported in COSMIC database) and novel variations, i.e., new mutations detected by NGS but not reported in either COSMIC or db SNP databases.
Outcome measures	Sensitivity and specificity
Outcome data	Detection of V600E BRAF mutation TN:32 TP:15 FN:2 FP:0

Study-level characteristics

	Study (N = 50)
Female	24%
Mean (range) age, *years*	62 (38-86) years

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(reference standard could employ either histology or cytology. However, diagnostic accuracy data is presented separately for each of these methods).*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low

Section	Question	Answer
Overall risk of bias and directness	**Risk of Bias**	**Low**
	Directness	**Directly applicable**

Ehsani, 2014

Bibliographic Reference Ehsani, Laleh; Cohen, Cynthia; Fisher, Kevin E; Siddiqui, Momin T; BRAF mutations in metastatic malignant melanoma: comparison of molecular analysis and immunohistochemical expression.; Applied immunohistochemistry & molecular morphology : AIMM; 2014; vol. 22 (no. 9); 648-51

Study Characteristics

Study type	Diagnostic accuracy study
Study details	Study location USA
Inclusion criteria	Metastatic malignant melanoma 19 excisional biopsies, and 6 fine-needle aspiration cell blocks.
Index test(s)	Immunohistochemistry IHC expression was evaluated by 2 different methods using the Dako autostainer (Abcam antibody) and Leica Bond Max (Spring Bioscience antibody), respectively. Antigen retrieval used an electric pressure cooker, at 15 to 20 pounds per square inch or 5 minutes at 1201C with cooling for 10 minutes before immunostaining. All tissues are then exposed to 3% hydrogen peroxide for 5 minutes, appropriately characterized and diluted primary antibody for 30 minutes, labeled polymer, HRP for 30 minutes, diaminobenzadine as chromogen for 5 minutes, and DAKO automation hematoxylin as counterstain for 15 minutes. These incubations are performed at room temperature; between incubations, sections are washed with Tris-Buffered Saline buffer. Cover slipping is performed using the Tissue-Tek SCA (Sakura Finetek USA Inc., Torrance, CA) automatic coverslipper. IHC analysis results were interpreted as positive if >10% of melanoma cells showed cytoplasmic staining of 2+ or 3+ intensity (Fig. 1). Molecular analysis was used as the gold standard for statistical analysis.
Reference standard (s)	COBAS 4800 PCR

	DNA specific Taq Man probes with different fluorescent dyes are directed atWT BRAF 600 (GTG sequence) and mutant BRAF V600E (GAG sequence). Following DNA template PCR amplification and mutant enrichment (if BRAF V600E mutation is present in the melanoma sample), the characteristic fluorescence is measured and the detection of BRAF V600E mutation is reported.
Sample size	25 samples
Outcome measures	Sensitivity and specificity
Outcome data	Detection of V600E BRAF mutation TN:7 TP:10 FN:0 FP:8

Study-level characteristics not reported

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear *(Unclear whether IHC was conducted blind to the results of the COBAS test)*
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(The study notes 8 FP results for IHC. Discordant cases did not undergo subsequent confirmatory testing.)*

Section	Question	Answer
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Moderate** ***(Unclear blinding and reference standard did not include exploration of discordant cases.)***
	Directness	**Directly applicable**

Fisher, 2014

Bibliographic Reference Fisher, Kevin E; Cohen, Cynthia; Siddiqui, Momin T; Palma, John F; Lipford, Edward H 3rd; Longshore, John W; Accurate detection of BRAF p.V600E mutations in challenging melanoma specimens requires stringent immunohistochemistry scoring criteria or sensitive molecular assays.; Human pathology; 2014; vol. 45 (no. 11); 2281-93

Study Characteristics

Study type	Diagnostic accuracy study retrospective study in which FFPE samples were tested with both IHC and COBAS
Study details	Study location USA Setting pathology department of hospital

	Study dates nr Sources of funding nr
Inclusion criteria	Malignant melanoma
Number of participants	124 samples, 118 were evaluated with both IHC and COBAS
Index test(s)	Immunohistochemistry Slides were loaded on the Ventana Bench Mark ULTRA (Ventana,Tucson,AZ) and tested with the OptiView diaminobenzidine (DAB) IHC Detection Kit(Ventana) per the package insert from the mutation-specific anti-BRAF V600E (VE1) mouse monoclonal antibody(Ventana) package insert. Briefly,slides were deparaffinized,condi- tioned for 64minutes,incubated with the BRAF V600E monoclonal antibody(3mg/mL) for 16 minutes at 36°C, and counterstained with hematoxyl in II for 4minutes. IHC score of 2+ or 3+ on at least 10% of cells was considered to be positive. Disagreement between the three pathologists was concluded through re-reviewing the samples together
Reference standard (s)	COBAS 4800 PCR DNA was isolated from one 5-μm section per sample (122 samples total) using the cobas DNA isolation kit according to the package insert. Microdissection of tumor-rich areas was not performed. Extracted sample DNA was tested with the cobas 4800 BRAF V600 Mutation Test kit (cobastest; Roche Molecular Systems, Branchburg, NJ).The cobas test is an FDA-approved real-time polymerase chain reaction (PCR) assay designed to detect the presence of the BRAF c.1799 TNA p.Val600Glu(p.V600 E) mutation in FFPE melanoma specimens. Discordant cases confirmed using NGS.
Outcome measures	Sensitivity and specificity
Outcome data	Detection of all V600 mutations *discrepant cases confirmed using NGS* TN:64 TP:41 FN:13 FP:0

Study-level characteristics

	Study (N = 118)
Primary melanoma *(%)*	46
metastatic melanoma *(%)*	50.8

	Study (N = 118)
>10% pigmentation *(%)*	33.9
>50% necrosis *(%)*	19.4
Core needle biopsy *(%)*	17.7
Tumor cells comprise <10% sample *(%)*	8.1

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear *(unclear blinding)*
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Unclear *(unclear blinding)*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low

Section	Question	Answer
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Moderate** ***(Unclear whether the index test, reference standard or confirmatory tests were conducted blind)***
	Directness	**Directly applicable**

Franczak, 2017

Bibliographic Reference Franczak, Claire; Salleron, Julia; Dubois, Cindy; Filhine-Tresarrieu, Pierre; Leroux, Agnes; Merlin, Jean-Louis; Harle, Alexandre; Comparison of Five Different Assays for the Detection of BRAF Mutations in Formalin-Fixed Paraffin Embedded Tissues of Patients with Metastatic Melanoma.; Molecular diagnosis & therapy; 2017; vol. 21 (no. 2); 209-216

Study Characteristics

Study type	Diagnostic accuracy study FFPE samples were taken from archive and prospectively tested
Study details	Study location France Setting Institut de Cance´rologie de Lorraine Tumour Bank, Vandoeuvre-le`s-Nancy, France Study dates Samples collected from 2011 to 2015 Sources of funding No funding

Inclusion criteria	Diagnosis of melanoma
Number of participants	59 samples, 2 samples from same person but collected from different metastases.
Index test(s)	Immunohistochemistry HC was assessed on 5 lm tissue sections from formalin-fixed, paraffin-embedded (FFPE) blocks, neighbouring the sections used for DNA islolation. The sections were deparaffinized with xylene and then rehydrated through a series of graded ethanol concentrations. VE1, a Val600Glu specific antibody (Spring Bioscience, Pleasanton, CA, USA) was used as previously described [20]. The Opti- View DAB IHC Detection Kit (Roche Diagnostics, Meylan, France) was used for visualization. The process was automated using BenchMark Ultra (Ventana, Meylan, France). Finally, staining localization was blindly assessed by a senior pathologist and scored using 0/1+, 2+ or 3+ notations. Staining was defined as 0 staining intensity when comparable to negative BRAF Val600Glu-negative control sample. Staining was defined as 0/1+, 2+ and 3+ for low, moderate and strong cytoplasmic staining intensities, respectively. Information which applies to all tests formalin-fixed paraffin-embedded tumour samples, with a minimal tumoral cells content of 10%
Reference standard (s)	COBAS 4800 PCR BRAF V600 mutations were assessed using the Cobas 4800 BRAF V600 CE-IVD mutation test kit (Roche Diagnostics, Meylan, France) and Cobas z480 thermocycler according to manufacturer's protocol. All data were automatically analyzed by the CE-IVD validated Cobas software (Roche Diagnostics). It was possible to used NGS to confirm discrepancies with IHC Next generation sequencing Ultra-deep pyrosequencing (GS Junior, Roche Diagnostics) was used for the detection of exon 15 mutations of BRAF. Fifty nanograms of DNA were used for PCR amplification. Specific primers were designed using Primer3Plus online software v.2.3.6. No other regions than exon 15 of BRAF have been enriched. Multiplex identifiers (MIDs), adaptations, and complementary sequence of the universal M13 tail were finally added to the first PCR products in a second PCR. Amplicon quality was assessed using 1% agarose gel. Amplicon processing was done as described by the Amplicon Library Preparation and emulsion PCR (emPCR; Lib-A) method GS junior titanium series manual from Roche Diagnostics. Amplicons were then purified using Agencourt AMPure XP beads (Beckman Coulter, SA, Nyon, Switzerland) and High Pure PCR Product purification kit (Roche Diagnostics). Quant-itTM PicoGreen dsDNA assay kit was used for DNA quantification (Life Technologies, Carlsbad, CA, USA). An emulsion PCR (emPCR) was finally assessed with 1 9 106 molecules. A total of 5 9 106 enriched beads were loaded on a picotiter plate for sequencing. Variant callings were assessed with the GS Amplicon Variant Analyzer (AVA, Roche Diagnostics) software version 3.0. A minimum of 1000 reads per amplicon per sample was required to validate the run for a 1% sensitivity and confirmed as previously described
Outcome measures	Sensitivity and specificity Compared to COBAS, V600, using NGS to confirm discrepancies: TN: 31 TP: 27 FN:1 FP:0 Compared to COBAS, V600, without confirmation: TN: 31 TP: 27 FN:1 FP:0 Compared to NGS, V600: TN: 28 TP: 27 FN:4 FP:0
Outcome data	Detection of all V600 mutations IHC vs COBAS, using NGS to confirm discrepancies: TN: 31 TP: 27 FN:1 FP:0 IHC vs COBAS, without confirmation: TN: 31 TP: 27 FN:1 FP:0 IHC vs NGS: TN: 28 TP: 27 FN:4 FP:0

Study-level characteristics

	Study (N = 59)
Primary tumours *(%)*	47.5
locoregional or distant metastases *(%)*	52.5

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Low**
	Directness	**Directly applicable**

Ihle, 2014

Bibliographic Reference Ihle, M. A., Fassunke, J., König, K., Grünewald, I., Schlaak, M., Kreuzberg, N., ... & Merkelbach-Bruse, S. (2014). Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p. V600E and non-p. V600E BRAF mutations. *BMC cancer*, *14*(1), 1-13.

Study Characteristics

Study type	Diagnostic accuracy study retrospective study in which FFPE samples were tested with IHC and either COBAS or NGS, or both
Study details	Study location Germany Setting Single centre Study dates 2010-2013 Sources of funding nr
Inclusion criteria	Melanoma
Number of participants	63 melanoma samples, 42 had recorded IHC results
Index test(s)	Immunohistochemistry Anti-BRAF p.V600E immunohistochemical staining was performed using the specific monoclonal mouse antibody VE1. Immunohistochemical staining was carried out within 2 weeks after cutting the 4 µm sections. Staining results were scored from 0 to 3+ by a senior pathologist (H. U. S. or I. G.) blinded to the results of molecular analysis. The staining was considered as positive for p.V600E staining (2+ and 3+) when the majority of viable tumor cells showed clear cytoplasmic staining. Negative staining results were interpreted when there

	was no or only slight staining, staining of only single cells or of monocytes and macrophages (0 and 1+).
Reference standard (s)	COBAS 4800 PCR DNA was isolated with the in-house method. Following the manufacturer's instructions, 5 ng/µl DNA of each sample were analyzed on the cobas z 480 system. If the concentration of the extracted DNA was too low, the maximum DNA volume of 25 µl was used. The results were displayed automatically as report by the cobas® z 480 software. NGS Targeted next generation sequencing (NGS) was performed on 72 FFPE samples. Isolated DNA (<0.5 – 97.6 ng/µl) was amplified with an in-house specified, customized Ion AmpliSeq Primer Pool. The panel comprises 102 amplicons of 14 different genes including exon 11 and 15 of the BRAF gene. PCR products were ligated to adapters and enriched for target regions using the Ion AmpliSeq PanelTM Library kit according to manufacturer's instructions (Life Technologies).
Outcome measures	Sensitivity and specificity
Outcome data	Detection of all V600 mutations *Compared to NGS* TN:5 TP:26 FN:8 FP:1 *Compared to COBAS* TN:7 TP:24 FN:2 FP:1 Detection of all V600e mutations *Compared to NGS* TN:13 TP:26 FN:0 FP:1

Study-level characteristics not reported

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low

Section	Question	Answer
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Low**
	Directness	**Directly applicable**

Lade-Keller, 2013

Bibliographic Reference	Lade-Keller J; Kristensen LS; Riber-Hansen R; Guldberg P; Hansen LL; Steiniche T; Hager H; A role for immunohistochemical detection of BRAF V600E prior to BRAF-inhibitor treatment of malignant melanoma?; Journal of clinical pathology; 2013; vol. 66 (no. 8)

Study Characteristics

Study type	Diagnostic accuracy study
Study details	Study location

	Denmark Setting All samples were used within 1 year of diagnosis and collected from the same laboratory Study dates samples collected over a 1 year period Sources of funding nr
Number of participants	28 samples
Index test(s)	Immunohistochemistry slides were pretreated with heat induced epitope retrieval (pH 9.0) using the Ventana Benchmark XT CC1S programme (Roche A/S, Hvidovre, Denmark). The antibody VE1 (dilution 1 : 40, Spring Bioscience, Pleasanton, California) was visualised by Ultraview fast red visualisation stain (Roche A/S, Hvidovre, Denmark). The intensity of the BRAF V600E protein stain was blindly and independently evaluated by two observers, one experienced dermatopathologist (evaluating samples twice) and one junior pathologist and categorised as either absent, uncertain or weak (0–1), or moderate to strong (2–3). Results from both observers were used for interobserver analysis, whereas results from the experienced pathologist alone were used for intraobserver analysis and comparison of mutational and IHC analysis.
Reference standard (s)	COBAS 4800 PCR Except for the preparation of the tissue (cutting the paraffin blocks and DNA extraction), all analyses were done according to the manufacturer protocol including dilution and standardization of the samples so that at least 125 ng DNA was used from each sample. Also used the mutation analysis Therascreen BRAF RGQ PCR kit (Qiagen, Manchester, UK) to specify the BRAF mutation subtype in samples with a weak IHC stain and with ambiguous mutational test results Samples had been tested in a previous study using COBAS, Sanger and Pyrosequencing, therefore confirmation of discrepant cases were possible using these methods.
Outcome measures	Sensitivity and specificity
Outcome data	Detection of V600E BRAF mutation with discrepant cases resolved using Sanger or pyrosequencing: TN:14 TP:13 FN:1 FP:0 Detection of all V600 mutations without discrepant cases resolved using Sanger or pyrosequencing: TN:14 TP:9 FN:1 FP:4

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	High *(2x2 data were possible using two reference standards: COBAS 4800 alone, this is a risk of bias as several results using this were found to be false positives, or COBAS plus discordant cases being confirmed using other methods used in the original study, this is also a risk of bias due as it is not reported whether these reference standards (Sanger sequencing or pyrosequencing) different in their result (it was only recorded whether one of them agreed with the IHC result). Additionally, this confirmation was not prospectively planned, and the single FN result recorded was not subject to this confirmation.)*

Section	Question	Answer
Overall risk of bias and directness	Risk of Bias	Moderate *(Both COBAS alone and COBAS with confirmatory testing are subject to risk of bias due to issues with the reference standards used [see flow and timing])*
	Directness	Directly applicable

Lo, 2016

Bibliographic Reference Lo, Michelle Chin I; Paterson, Anna; Maraka, Jane; Clark, Richard; Goodwill, Joseph; Nobes, Jenny; Garioch, Jennifer; Moncrieff, Marc; Rytina, Ed; Igali, Laszlo; A UK feasibility and validation study of the VE1 monoclonal antibody immunohistochemistry stain for BRAF-V600E mutations in metastatic melanoma.; British journal of cancer; 2016; vol. 115 (no. 2); 223-7

Study Characteristics

Study type	Diagnostic accuracy study Retrospective analysis of data that were recorded during a prospective audit
Study details	Study location UK Setting Addenbrooke's and Norfolk and Norwich University hospitals databases Study dates Unclear Sources of funding The VE1 antibody was acquired in Norwich using a grant from the Skin Cancer Research Fund.
Inclusion criteria	Diagnosis of melanoma

	Tested for BRAF mutation All melanoma samples sent away for BRAF V600 mutation testing using the COBAS technique were identified from our respective electronic records databases held in the pathology departments. Cases with only BRAF IHC or genomic analysis were excluded.
Number of participants	219 samples from 214 patients.
Index test(s)	Immunohistochemisty IHC on paraffin-embedded samples using the VE1 monoclonal antibody (Spring Bioscience, Pleasanton, CA, USA) was undertaken prospectively by the in-house pathology services and reported by the local sub-specialty pathologists before the sample being sent for genomic analysis.
Reference standard (s)	COBAS 4800 PCR Mutation testing was undertaken at a national molecular testing centre (Birmingham) using the COBAS technique. In three cases from Addenbrooke's hospital where IHC suggested the presence of the V600E mutation and the COBAS test was negative, pyrosequencing of BRAF was undertaken by the reference centre. The further genomic analysis was specifically requested in these cases by the local MDT to verify the reason for the false-negative COBAS test, since during the early part of the study a positive molecular result was required for the patient to receive treatment with a BRAF inhibitor. No further genomic analysis was undertaken in cases which were negative by IHC but positive on the COBAS test,since it is known that IHC only detects V600E mutations.
Outcome measures	Sensitivity and specificity 2x2 table for diagnostic accuracy of IHC relative to PCR for people with stage III melanoma. FN: 5 FP: 5 TN: 80 TP: 62
Outcome data	Detection of all V600 mutations With discrepant cases confirmed using pyrosequencing*: TN: 124 TP: 87 FN: 5 FP: 3 * only 3/11 discrepant cases were confirmed using pyrosequencing Without discrepant cases confirmed using pyrosequencing: TN: 124 TP: 84 FN: 5 FP: 6

Population characteristics not reported

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	High *(The study was a prospective audit however the author notes that cases in which the participants received only one test (IHC or COBAS) were excluded. It is not clear whether this led to selection bias in which participants with a negative IHC were likely to be included in the study [as these participants are more likely to subsequently undergo COBAS testing]).*
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear (Unclear whether index tests were interpreted blind)
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(Author notes that in 3 cases the IHC was positive but the COBAS test was negative, with subsequent pyrosequencing confirming that the IHC was correct and the results on the COBAS test were false negatives. Not all discrepant cases underwent confirmatory imaging)*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	High
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Moderate** ***(Unclear blinding procedures. Not all participants underwent confirmatory imaging).***

Section	Question	Answer
	Directness	Directly applicable

Nielsen, 2018

Bibliographic Reference	Nielsen, Line B; Dabrosin, Nina; Sloth, Karen; Bonnelykke-Behrndtz, Marie L; Steiniche, Torben; Lade-Keller, Johanne; Concordance in BRAF V600E status over time in malignant melanoma and corresponding metastases.; Histopathology; 2018; vol. 72 (no. 5); 814-825

Study Characteristics

Study type	Diagnostic accuracy study patients were prospectively evaluated with both tests.
Study details	Study location Denmark Setting Aarhus University Hospital Study dates 1 January 2011 to 1 August 2014, Sources of funding nr
Inclusion criteria	Metastatic melanoma
Number of participants	314 samples, 224 included in analysis.
Loss to follow-up	90 participants could not be evaluated due to insufficient sample material

Index test(s)	Immunohistochemistry IHC staining was performed on the same FFPE material that was used for mutation testing. 3-lm section was cut from each tissue block, mounted on Superfrost Plus slides (Thermo Fisher Scientific, Waltham, MA, USA), and dried for 1 h at 60°C. Immunohistochemistry was performed on the BenchMark XT. Monoclonal mouse antibody against BRAF V600E was incubated for 32 min at room temperature, and this was followed by detection with the Ventanas ultraView Universal Alkaline Phosphatase Red Detection Kit and signal amplification. Intensity was judged on a scale of 0 to 3 (Figure 2): no staining (0), very weakly positive staining ("), weakly positive staining (1), moderately positive staining (2), and strongly positive staining (3). In any discordant cases, the two observers viewed the slides together to reach an agreement.
Reference standard (s)	COBAS 4800 PCR Cobas 4800 System, v2.0 designed to detect the BRAF V600E (1799 T>A) mutation. The results were scored in a binary fashion as 'mutated' or 'wild-type' BRAF V600. Reanalysis of patients in whom findings of the Cobas PCR analysis and the VE1 IHC analysis were discordant was performed with the Therascreen BRAF RGQ PCR kit (Qiagen, Manchester, UK). This is an in-vitro diagnostic test for detection of somatic mutations found in BRAF [V600E (c.1799 T>A), V600Ec (c.1799_1800 TG>AA), V600D (c.1799_1800 TG>AT), V600R (c.1798_1799 GT>AG), and V600K (c.1798_1799 GT>AA)].
Outcome measures	Sensitivity and specificity
Outcome data	Detection of V600E BRAF mutation where discordant cases were assessed using Quiagen gold standard: TN:121 TP:105 FN:1 FP:0 Detection of all V600 mutations where discordant cases were assessed using Quiagen gold standard: TN:112 TP:105 FN:10 FP:0 where discordant cases were not subject to further testing: TN:112 TP:100 FN:10 FP:5

Population characteristics

	Study (N = 224)
Female	
BRAF V600e mutated *(%)*	58
BRAF V600e wild-type *(%)*	61
Mean age (SD)	
BRAF V600e mutated *Mean (SD) years*	56 (14)

	Study (N = 224)
BRAF V600e wild-type *Mean (SD) years*	67 (11)
Ulceration present	
BRAF V600e mutated *(%)*	32
BRAF V600e wild-type *(%)*	28

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	High *(83 participants were excluded due to insufficient material on the sample to conduct an IHC test. 90 additional samples were excluded due to not having a sample available in archive. It is unclear whether these exclusions lead to selection bias.)*
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low

Section	Question	Answer
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	Risk of Bias	Low
	Directness	Directly applicable

O'Brien, 2017

Bibliographic Reference O'Brien, Odharnaith; Lyons, Tomas; Murphy, Sandra; Feeley, Linda; Power, Derek; Heffron, Cynthia C B B; BRAF V600 mutation detection in melanoma: a comparison of two laboratory testing methods.; Journal of clinical pathology; 2017; vol. 70 (no. 11); 935-940

Study Characteristics

Study type	Diagnostic accuracy study Using samples which previously had BRAF mutation status determined using COBAS test.
Study details	Study location Ireland Setting Single centre Study dates patients diagnosed with metastatic melanoma in our institution from 2012 to 2014. Samples were subsequently tested using IHC Sources of funding None
Inclusion criteria	Metastatic melanoma

	availability of formalin fixed paraffin embedded (FFPE) tissue blocks with sufficient material for IHC staining following molecular analysis
Number of participants	132 samples; 122 included in analysis
Index test(s)	Immunohistochemistry Immunohistochemistry for the BRAFV600E mutation was performed using the Roche Ventana anti-BRAFV600E VE1 clone antibody on the Ventana Benchmark Ultra platform slide staining system. The process involved cell conditioning for 64 min, preoxidation inhibition and primary antibody incubation for 16 min at 36°C. Ventana Optiview 3'3 – Diaminobenzidine (DAB) IHC detection kit was used to detect BRAFV600E protein expression. The slides were counterstained with Ventana haematoxylin and Bluing agent for 4 min. Specimens deemed to have excessive melanin pigment deposits as determined from the original H&E slides were grouped together in the TMA blocks and were analysed for the BRAFV600E mutation using a Ventana Alkaline Phosphatase Red Detection Kit. A scoring scale of 0–3 was used, with strong cytoplasmic staining scored as 3+, medium cytoplasmic staining as 2+, weak cytoplasmic staining as 1+ and the absence of staining as 0.
Reference standard (s)	COBAS 4800 PCR BRAF status was on record. All participants underwent COBAS 4800 PCR. One participant had status confirmed using NGS, it is unclear whether any other participants underwent additional screening
Outcome measures	Sensitivity and specificity Mutation status was determined from record. All participants with FN IHC results responded to BRAF inhibitors suggesting actual true positive. additional evaluation with NGS determined V600K mutation in one participant.
Outcome data	Detection of all V600 mutations TN:87 TP:29 FN:5 FP:1

Population characteristics

	Study (N = 122)
Female *(%)*	52.3
Median (Range) age *(years)*	61 (16 to 94)

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Low
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(Participants initially underwent COBAS testing however their BRAF status was taken from their record, it is unclear whether participants underwent a standardised testing procedure or whether these results are solely the results of the COBAS.)*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	High *(One participant underwent subsequent testing using NGS however it is unclear whether other participants did also. Therefore, the data from this study was treated as being determined by COBAS alone).*
Overall risk of bias and directness	**Risk of Bias**	**High** ***(Unclear protocol for determining BRAF status. Discordant cases likely did not all undergo confirmatory analysis)***
	Directness	**Directly applicable**

Schirosi, 2016

Bibliographic Reference Schirosi, Laura; Strippoli, Sabino; Gaudio, Francesca; Graziano, Giusi; Popescu, Ondina; Guida, Michele; Simone, Giovanni; Mangia, Anita; Is immunohistochemistry of BRAF V600E useful as a screening tool and during progression disease of melanoma patients?.; BMC cancer; 2016; vol. 16 (no. 1); 905

Study Characteristics

Study type	Diagnostic accuracy study Retrospective study
Study details	Study location Italy Setting Patients at Istituto Tumori "Giovanni Paolo II" Study dates June 2008 to April 2015 Sources of funding no funding
Inclusion criteria	Metastatic melanoma 5 primary melanoma, 21 lymph node metastases, 25 subcutaneous metastases, 13 other anatomical sites
Number of participants	64 participants
Index test(s)	Immunohistochemistry Ventana® BRAF V600E (VE1) mouse monoclonal primary antibody on the Ventana® Benchmarck XT automated slide strainer in combination with the Ventana OptiView DAB IHC Detection Kit: IHC was performed on the same formalin-fixed, paraffin-embedded tissue block used for mutational testing and the histological sections of the relative samples were reviewed by a pathologist to assure the presence of a sufficient tumor content. All immunoreactive samples were scored by double-blinded independent observers who had no information on patient clinical and molecular data. The slides were scored as positive when more than 90% of tumor cells showed a clear moderate to strong brown cytoplasmic staining, while they were considered negative when there was no staining or only nuclear dot staining, weak staining of single interspersed cells, or staining of monocytes/macrophages. Secondly the intensity of immunostaining was graded 0 if there was no visible staining, grade 1 if weak diffuse cytoplasmic background staining was

	present, grade 2 if moderate diffuse and granular cytoplasmic staining was observed and grade 3 if strong mainly granular cytoplasmic staining was detected. No staining (grade 0) and staining grade 1 were regarded as negative for V600E, while grade 2 and grade 3 were regarded as positive samples.
Reference standard (s)	COBAS 4800 29 cases tested using the cobas® BRAF V600 test.
Outcome measures	Sensitivity and specificity
Outcome data	Detection of all V600 mutations TN: 4 TP: 23 FN: 2 FP: 0

Population characteristics

	Study (N = 64)
Female *(%)*	45.3
Median (Range) age *(years)*	61 (22 to 82)
AJCC stage at diagnosis *(%)*	
I	1.6
II	30.1
III	41.3
IV	27
Brain metastasis *(%)*	35.9

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	High
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(the study included all participants who underwent COBAS 4800, across a wide time period (June 2008 to April 2015). Methodology for conducting COBAS test was not reported and most likely different over time. Discordant cases between IHC and COBAS did not undergo subsequent testing for confirmation (Although this only applied to two cases).)*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**High** ***(Unclear methodology for conducting COBAS 4800. Study used samples which were tested over a 7 year period and it is likely that the way in which COBAS 4800 was used differed over this time. Discrepant cases were not confirmed using subsequent testing.)***
	Directness	**Directly applicable**

Sener, 2017

Bibliographic Reference	Sener, Ebru; Yildirim, Pinar; Tan, Ayca; Gokoz, Ozay; Tezel, Gaye Guler; Investigation of BRAF mutation analysis with different technical platforms in metastatic melanoma.; Pathology, research and practice; 2017; vol. 213 (no. 5); 522-530

Study Characteristics

Study type	Diagnostic accuracy study using Formaldehyde fixed-paraffin-embedded (FFPE) tissue blocks
Study details	Study location Turkey Setting Single pathology department Study dates nr Sources of funding supported by Hacettepe University Scientific Research Projects Coordination Unit (No: 013D11101004-409)
Inclusion criteria	Metastatic melanoma
Number of participants	98 patients. The tissue samples in 72 cases (73.5%) were obtained from the primary tumour and from the metastatic tumour in 26 cases (26.5%).
Index test(s)	Immunohistochemistry using the anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody. The results of the IHC were classified as positive, negative, equivocal and not assessed. In this case, positive showed moderate intensity of cytoplasmic staining, while negative showed no staining, diffuse poor cytoplasmic staining, isolated nuclear staining and monocyte-macrophage staining. Equivocal results showed weak and non-specific staining, while not assessed results showed no tumour in the paraffin block and/or on the IHC slide. Information which applies to all tests All samples contained at least 50% tumour tissue. For each sample, DNA concentration was measured with a NanoDrop 200 UV–vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 260 nm. The DNA samples obtained from the patient’s tumour tissue were used for both molecular methods.

Reference standard (s)	COBAS 4800 PCR cobas 4800 BRAF V600 Mutation Test: The DNA sample was mixed on the plates and loaded into the cobas 4800 System v2 analyzer for mutation analysis. The results were processed by the BRAF mutation analysis software as mutation detected or mutation not detected. Discordant cases were confired using pyrosequencing for the purpose of this study
Outcome measures	Sensitivity and specificity
Outcome data	Detection of all V600 mutations using pyrosequencing to confirm discordant cases: TN:67 TP:24 FN:3 FP:0 v600, using pyrosequencing to confirm discordant cases: TN:64 TP:22 FN:6 FP:2

Population characteristics

	Study (N = 98)
% Female	48
Median (IRQ) age *(years)*	59.6 (24 to 87)

Risk of bias

Section	**Question**	**Answer**
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear *(Unclear whether the tests were interpreted blind to the results of the other tests.)*
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low

Section	Question	Answer
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Low
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Moderate** ***(unclear whether tests were conducted blind.)***
	Directness	**Directly applicable**

Tetzlaff, 2015

Bibliographic Reference Tetzlaff, Michael T; Pattanaprichakul, Penvadee; Wargo, Jennifer; Fox, Patricia S; Patel, Keyur P; Estrella, Jeannelyn S; Broaddus, Russell R; Williams, Michelle D; Davies, Michael A; Routbort, Mark J; Lazar, Alexander J; Woodman, Scott E; Hwu, Wen-Jen; Gershenwald, Jeffrey E; Prieto, Victor G; Torres-Cabala, Carlos A; Curry, Jonathan L; Utility of BRAF V600E Immunohistochemistry Expression Pattern as a Surrogate of BRAF Mutation Status in 154 Patients with Advanced Melanoma.; Human pathology; 2015; vol. 46 (no. 8); 1101-10

Study Characteristics

Study type	Diagnostic accuracy study retrospective review of tests over a two year period
Study details	Study location USA Setting

	Patients treated at MD Anderson Study dates January 1, 2011 to January 31, 2013 Sources of funding None
Inclusion criteria	Diagnosis of melanoma
Number of participants	154
Index test(s)	Immunohistochemistry IHC staining with anti-BRAF V600E (clone VE1) was performed on matched tumor samples submitted for molecular testing. Clone VE1 to BRAF V600E (Spring Bioscience) was used at a 1:50 dilution with an automated IHC staining instrument. Intensity of staining was also recorded: weak, moderate, and strong.
Reference standard (s)	Next generation sequencing BRAF mutation testing was performed on an Ion Torrent Personal Genome Machine (IT-PGM) 46/50 cancer-related gene NGS platform in the CLIA-certified MDL at our institution
Outcome measures	Sensitivity and specificity Negative and weak counted as negative, all others counted positive
Outcome data	Detection of V600E BRAF mutation TN: 97 TP: 52 FN: 1 FP: 4 Detection of all V600 mutations BRAF non-V600 counted as negative. TN: 97 TP: 55 FN: 1 FP: 1

Population characteristics

	Study (N = 154)
Female *(%)*	35

	Study (N = 154)
IHC expression pattern	
Negative *(%)*	64
Heterogenous *(%)*	6
Homogenous *(%)*	30
IHC staining intensity	
Negative *(%)*	64
Weak *(%)*	5
Moderate *(%)*	5
Strong *(%)*	27
Median (Range) age *(years)*	61 (26 to 87)

Risk of bias

Section	Question	Answer
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear

Section	Question	Answer
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Unclear
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	Unclear
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Unclear
Flow and timing: risk of bias	Could the patient flow have introduced bias?	Low
Overall risk of bias and directness	**Risk of Bias**	**Moderate** ***(Unclear whether tests were interpreted blind to each other)***
	Directness	**Directly applicable**

Uguen, 2015

Bibliographic Reference Uguen, Arnaud; Gueguen, Paul; Legoupil, Delphine; Bouvier, Stephanie; Costa, Sebastian; Duigou, Sandrine; Lemasson, Gilles; Lede, Francoise; Sassolas, Bruno; Talagas, Matthieu; Ferec, Claude; Le Marechal, Cedric; De Braekeleer, Marc; Marcorelles, Pascale; Dual NRASQ61R and BRAFV600E mutation-specific immunohistochemistry completes molecular screening in melanoma samples in a routine practice.; Human pathology; 2015; vol. 46 (no. 11); 1582-91

Study Characteristics

Study type	Diagnostic accuracy study
Study details	Study location France

	Setting specimens of melanomas sent to the Brest University Hospital cancer molecular genetic platform Study dates January to December 2014 Sources of funding None reported
Number of participants	111; 104 included in analysis
Loss to follow-up	6 results were inconclusive on reference standard and 1 inconclusive on IHC
Index test(s)	Immunohistochemistry BRAF V600E (clone VE1; Spring Bioscience) at a dilution of 1:100 were used. IHC was performed on Ventana Benchmark XT automated slide preparation system using ultraView Universal Alkaline Phosphatase Red Detection Kit.
Reference standard (s)	Composite Pyrosequencing was used as the primary reference standard. Discordant cases in which the result on pyrosequencing was negative but IHC was positve underwent secondary IHC in addition to NGS to determine actual mutation status. Next generation sequencing DNA libraries were produced using custom Ion AmpliSeq Panel (Life Technologies [LT], Saint-Aubin, France) according to the manufacturer's instruction. After library quantification by quantitative polymerase chain reaction (Ion Library Quantitation Kit; LT) and Roche 480 Lightcycler Real-Time PCR (Roche Diagnostics, Meylan, France), 15 bar-coded (Ion Xpress Barcodes adapters Kit; LT) tumor DNA libraries were sequenced simultaneously on a 316 chip in the Personal Genome Machine system (Ion Torrent; LT). Torrent suite software version 4.4.0 was used for signal processing, running quality report, and Fastq file generation. BRAF and NRAS sequences were then analyzed through the SeqNext software version 4.1.2 (JSI medical systems, Ettenheim, Germany) (custom AmpliSeq design and parameters available upon request). Pyrosequencing amplified using the multiplex PCR kit (Qiagen, Courtaboeuf, France) in a 20-µL final volume containing 2 µL of the tumor DNA. Genotyping of codon 600 of BRAF and codon 61 of NRAS was carried out on PyroMark Q24 system (Qiagen)
Outcome measures	Sensitivity and specificity Doubtful and negative results were treated as negative.
Outcome data	Detection of V600E BRAF mutation discordant cases confirmed using NGS: TN: 80 TP: 24 FN: 0 FP: 0 Detection of all V600 mutations

	discordant cases confirmed using NGS: TN: 71 TP: 24 FN: 9 FP: 0

Population characteristics not presented

Risk of bias

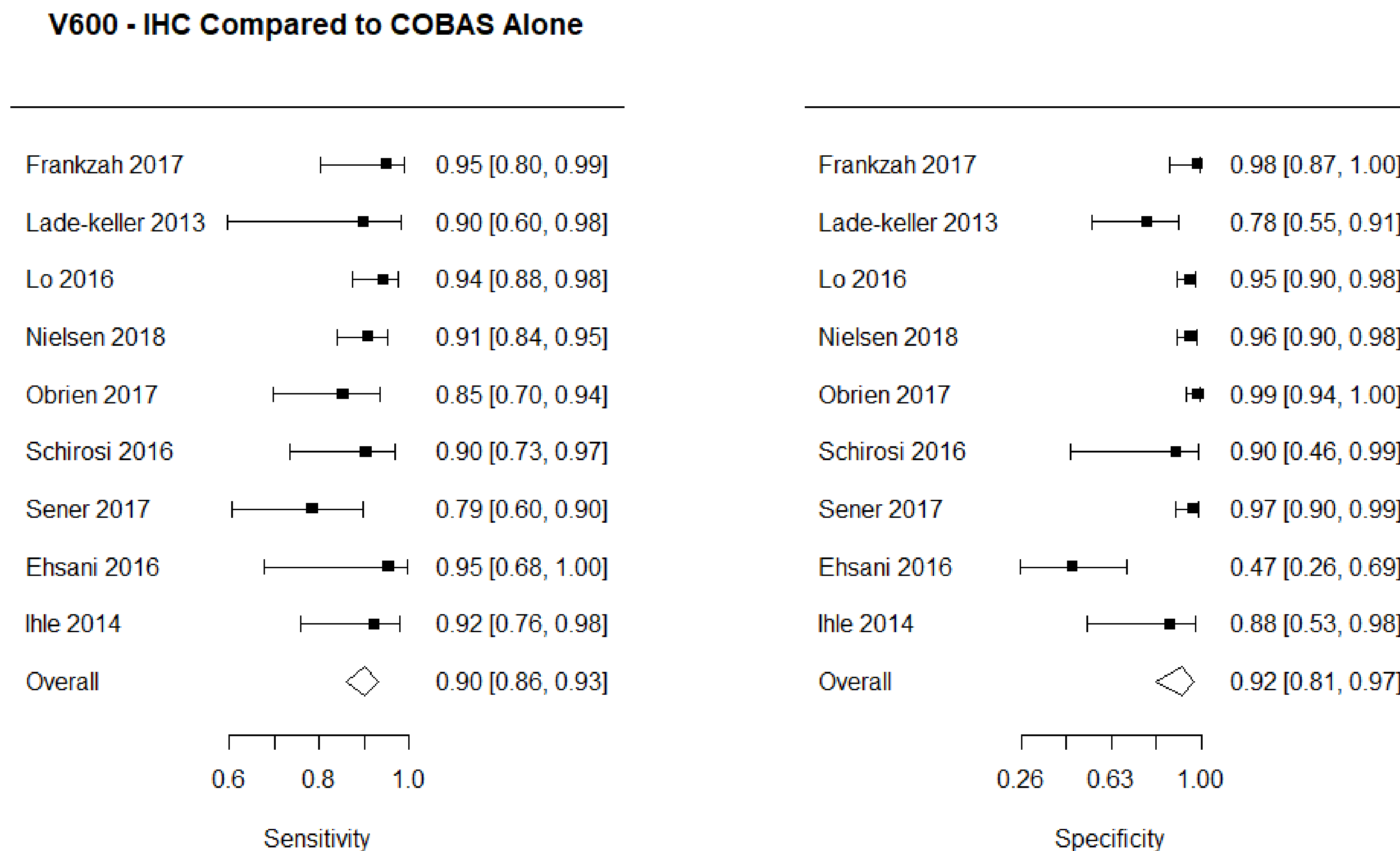
Section	**Question**	**Answer**
Patient selection: risk of bias	Could the selection of patients have introduced bias?	Low
Patient selection: applicability	Are there concerns that included patients do not match the review question?	Low
Index tests: risk of bias	Could the conduct or interpretation of the index test have introduced bias?	Unclear
Index tests: applicability	Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low
Reference standard: risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?	High *(sensitivity of pyrosequencing is not as reliable as NGS. However, v600e discordant cases underwent confirmatory testing using NGS)*
Reference standard: applicability	Is there concern that the target condition as defined by the reference standard does not match the review question?	Low
Flow and timing: risk of bias	Could the patient flow have introduced bias?	High *(Only discordant cases underwent NGS as a secondary reference standard. Ideally, all participants would have undergone NGS which is a more sensitive method of detecting NGS)*
Overall risk of bias and directness	**Risk of Bias**	**High** ***(Unclear blinding procedures. Pyrosequencing less sensitive than NGS.***

Section	Question	Answer
		Discordant cases were confirmed using NGS but only for discordant v600e cases.)
	Directness	**Directly applicable**

Appendix E – Forest plots

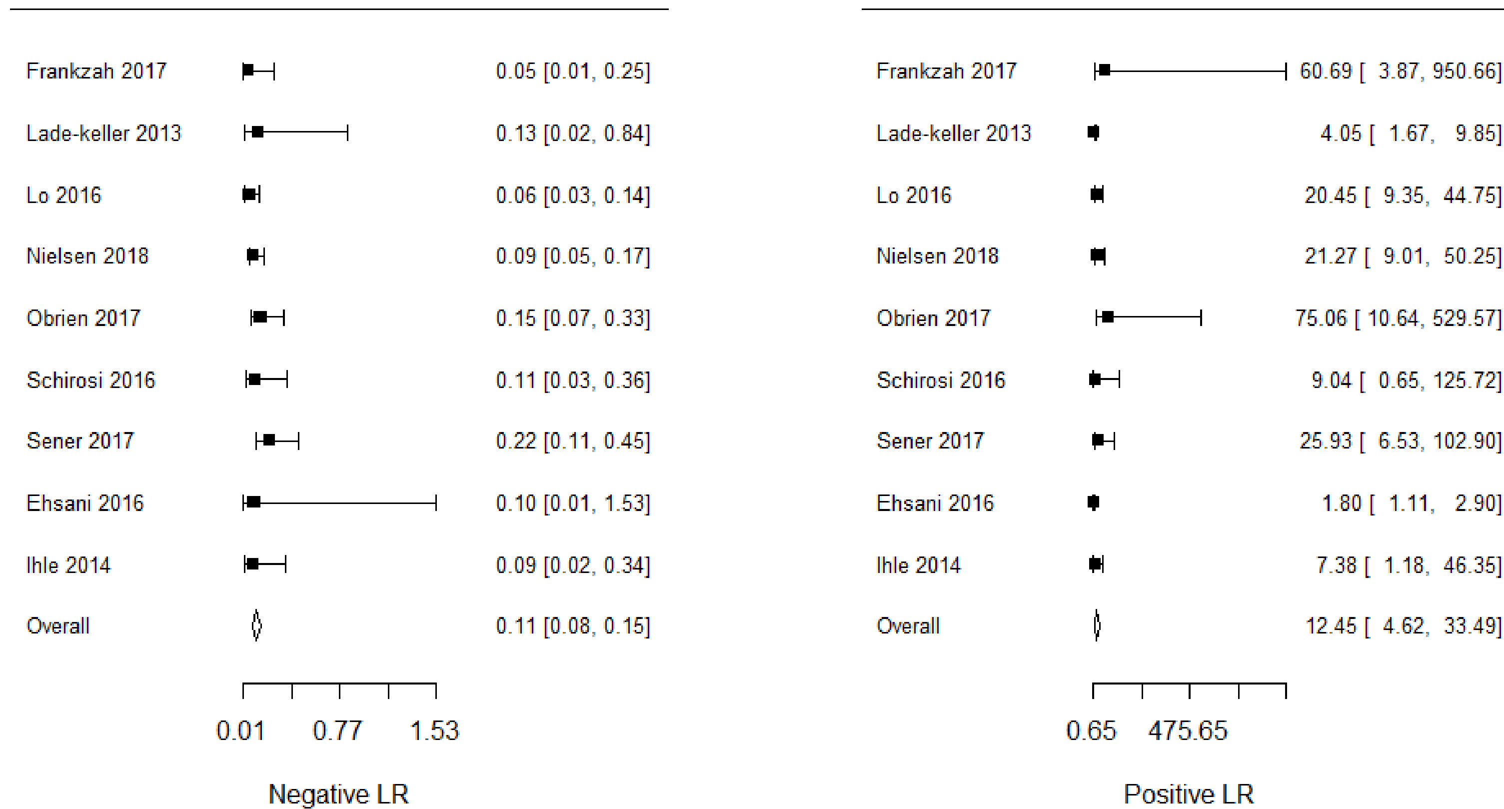
meta-analyses were performed for this review.

Figure 1: Sensitivity and specificity for IHC compared to COBAS alone for the detection of any V600 mutation



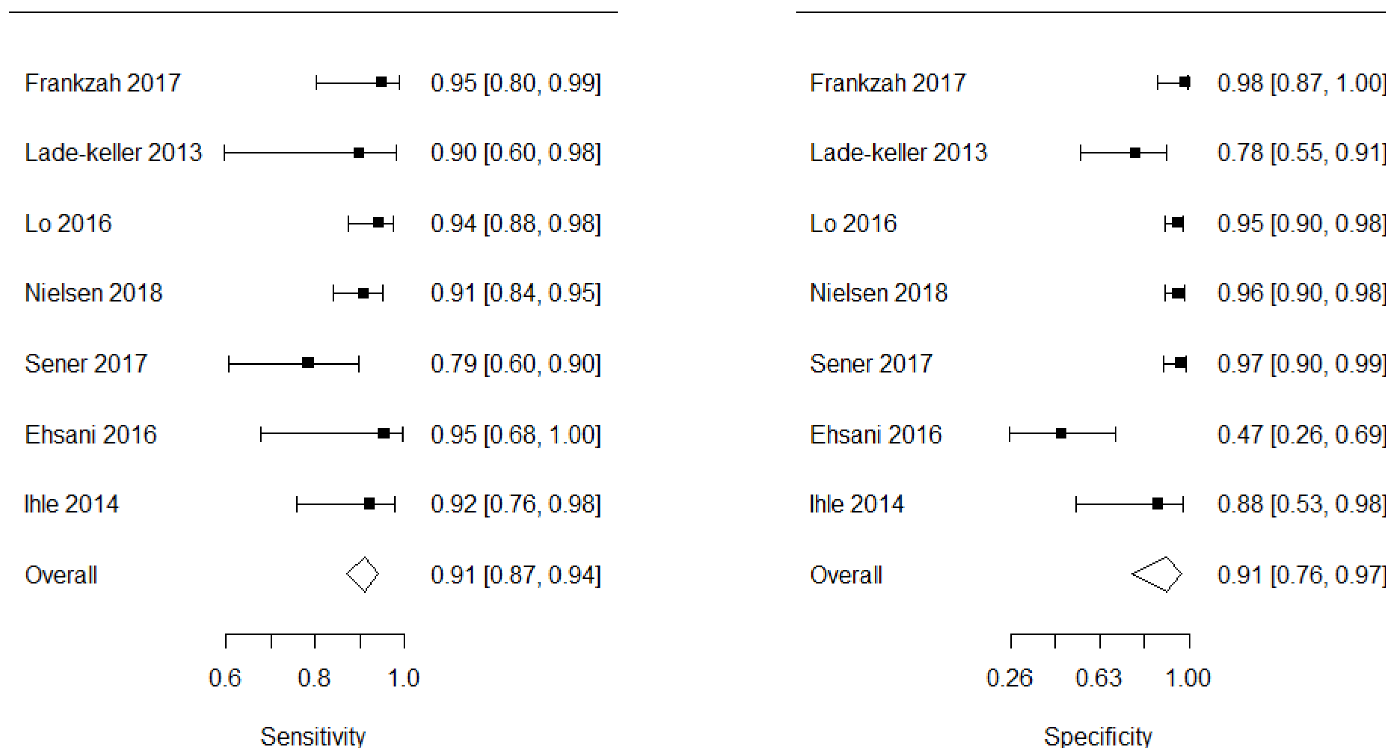
Sensitivity I^2= 0.0%, Specificity I^2= 81.0%

Figure 2: Likelihood ratios for IHC compared to COBAS alone for the detection of any V600 mutation



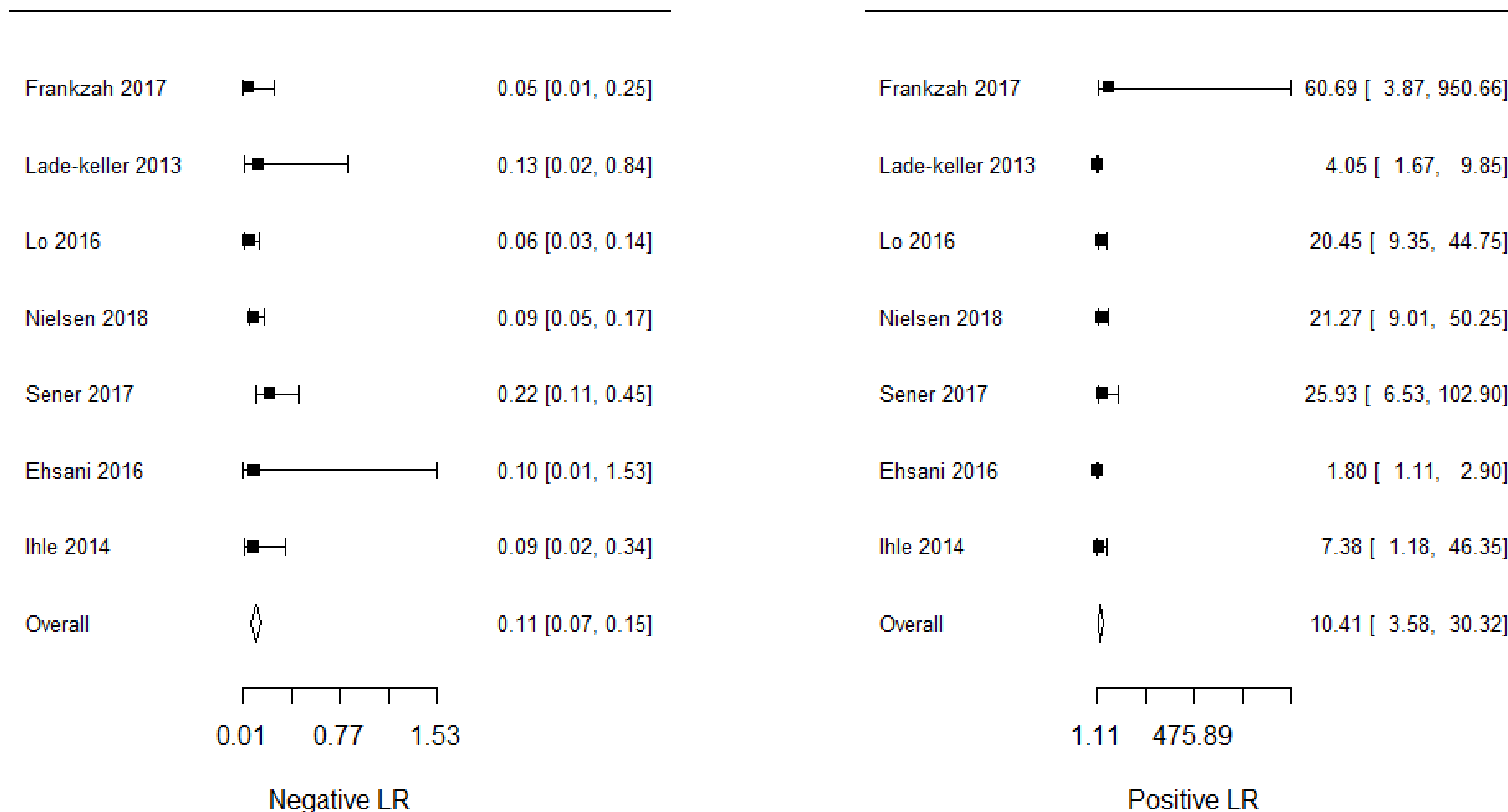
Positive likelihood ratio I^2= 85.9%, negative likelihood ratio I^2= 0.0%

Figure 3: Sensitivity and specificity for IHC compared to COBAS alone for the detection of any V600 mutation, excluding high risk of bias studies



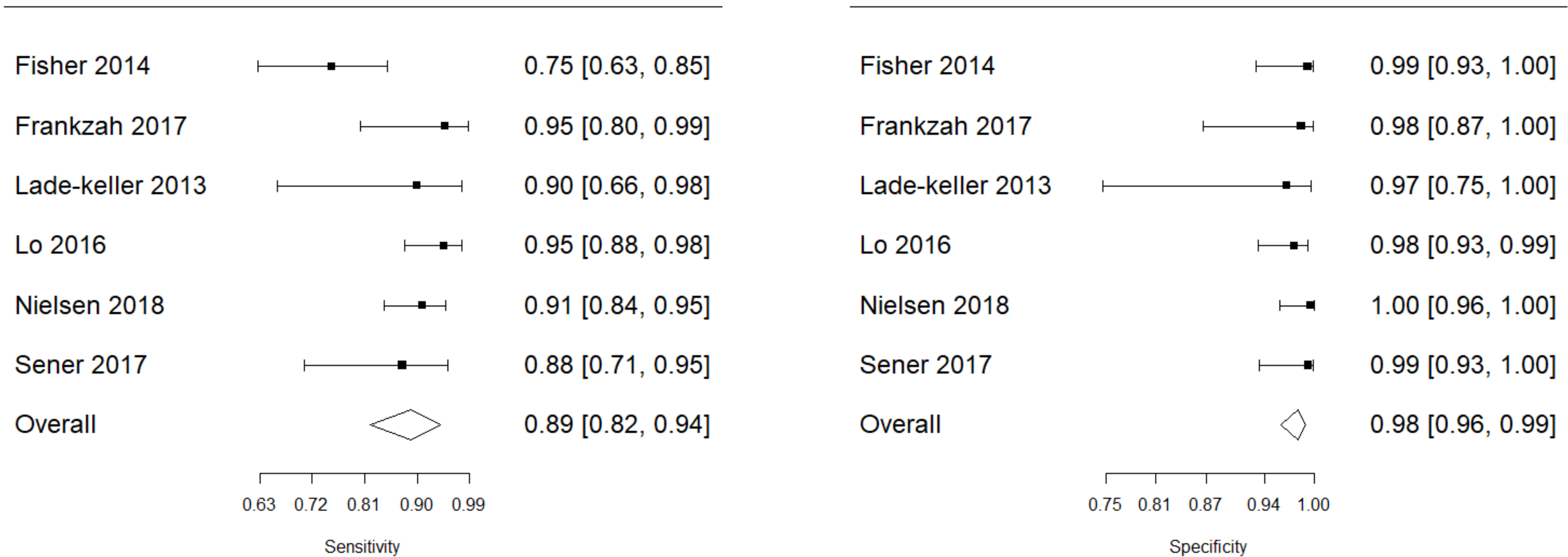
Sensitivity I^2= 12.3%, Specificity I^2= 83.9%

Figure 4: Likelihood ratios for IHC compared to COBAS alone for the detection of any V600 mutation, excluding high risk of bias studies



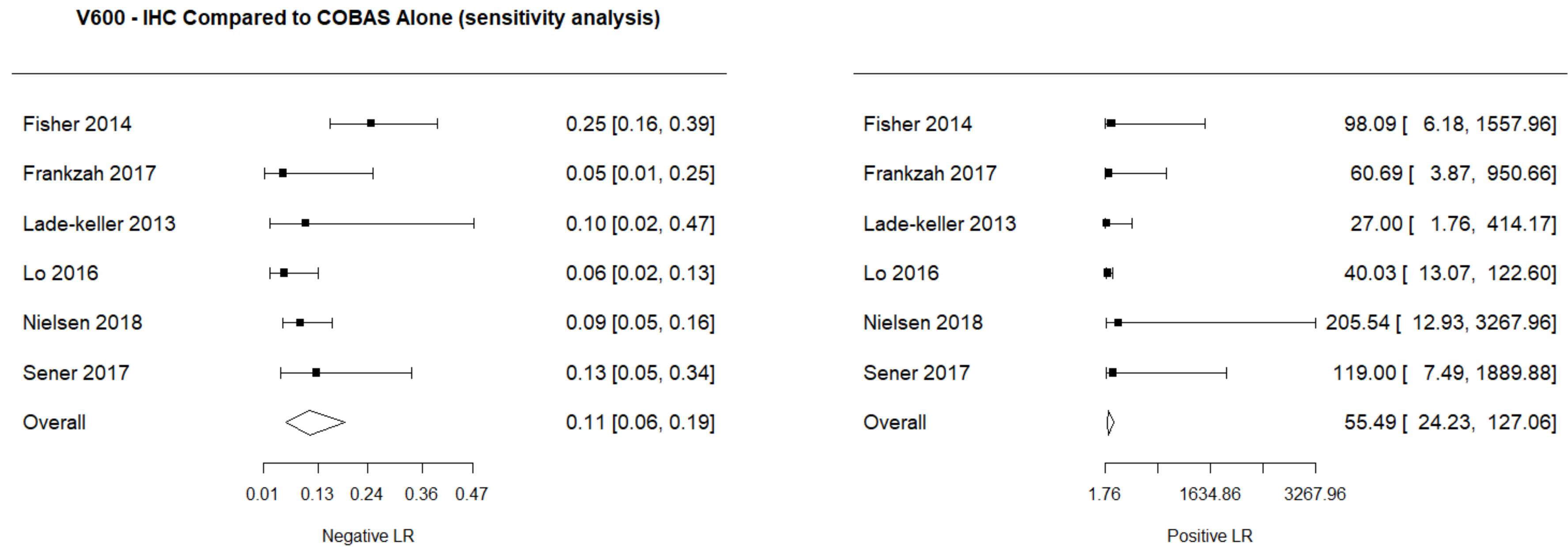
Positive likelihood ratio I^2= 87.9%, negative likelihood ratio I^2= 13.8%

Figure 5: Sensitivity and specificity for IHC compared to COBAS for the detection of any V600 mutation, with discrepancies confirmed using third testing method



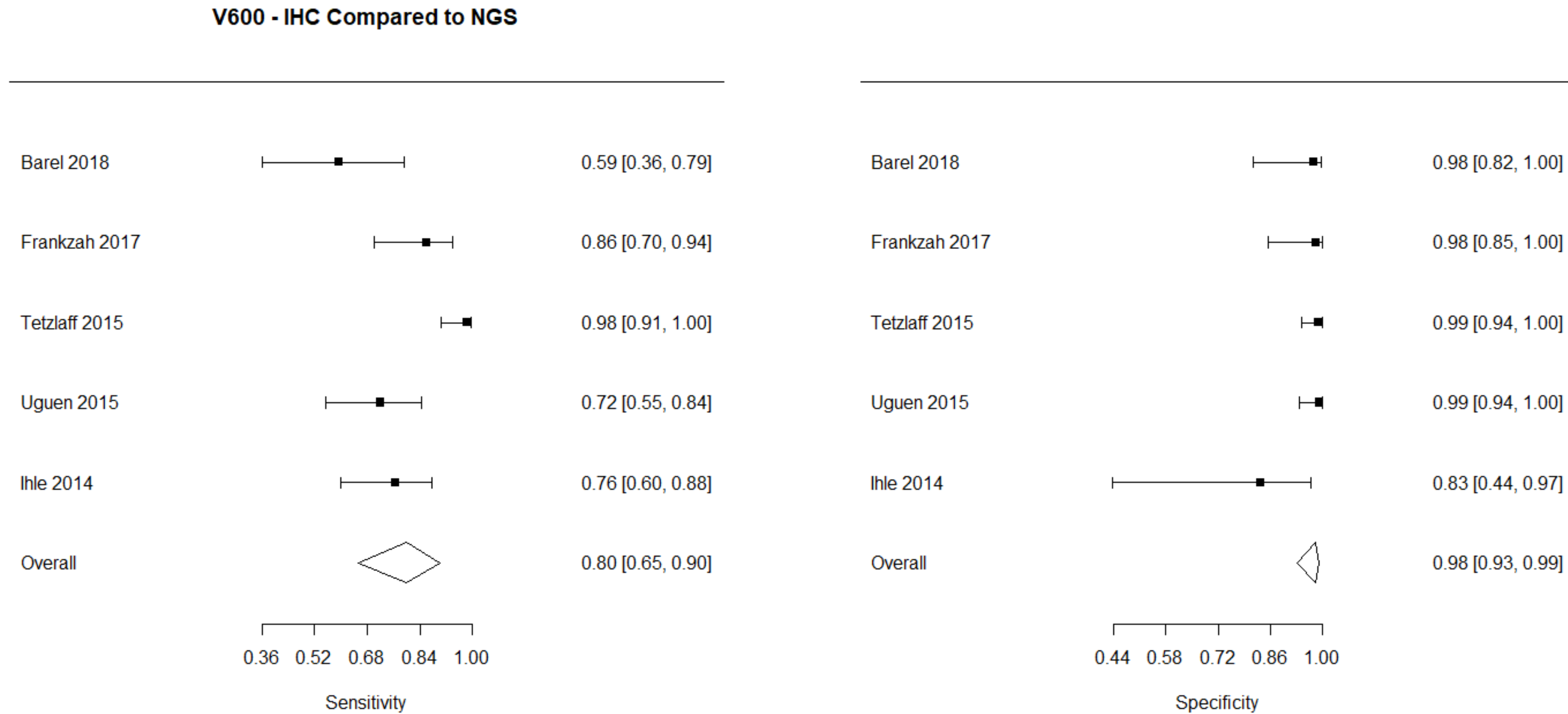
Sensitivity I^2= 62.8%, Specificity I^2= 0.0%

Figure 6: Likelihood ratios for IHC compared to COBAS for the detection of any V600 mutation, with discrepancies confirmed using third testing method



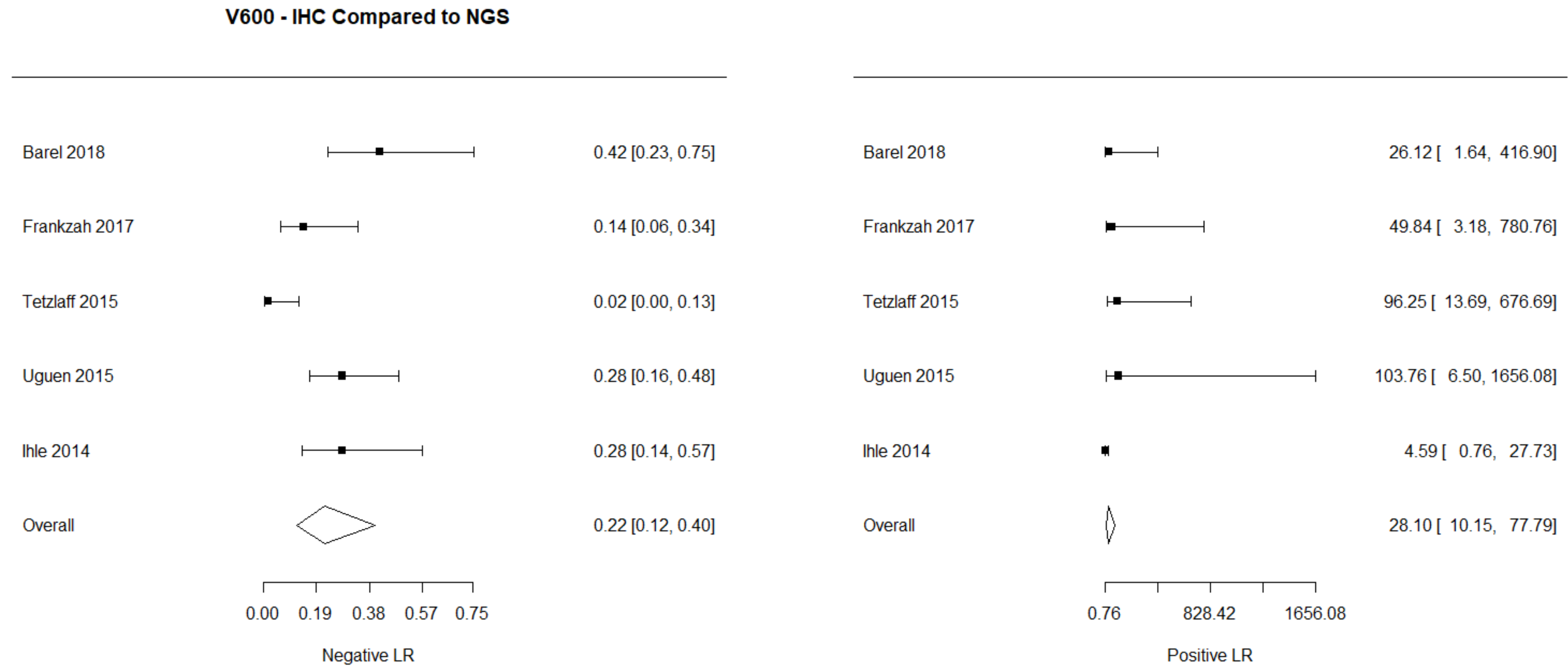
Positive likelihood ratio I^2= 0.0%, negative likelihood ratio I^2= 64.4%

Figure 7: Sensitivity and specificity for IHC compared to NGS for the detection of any V600 mutation



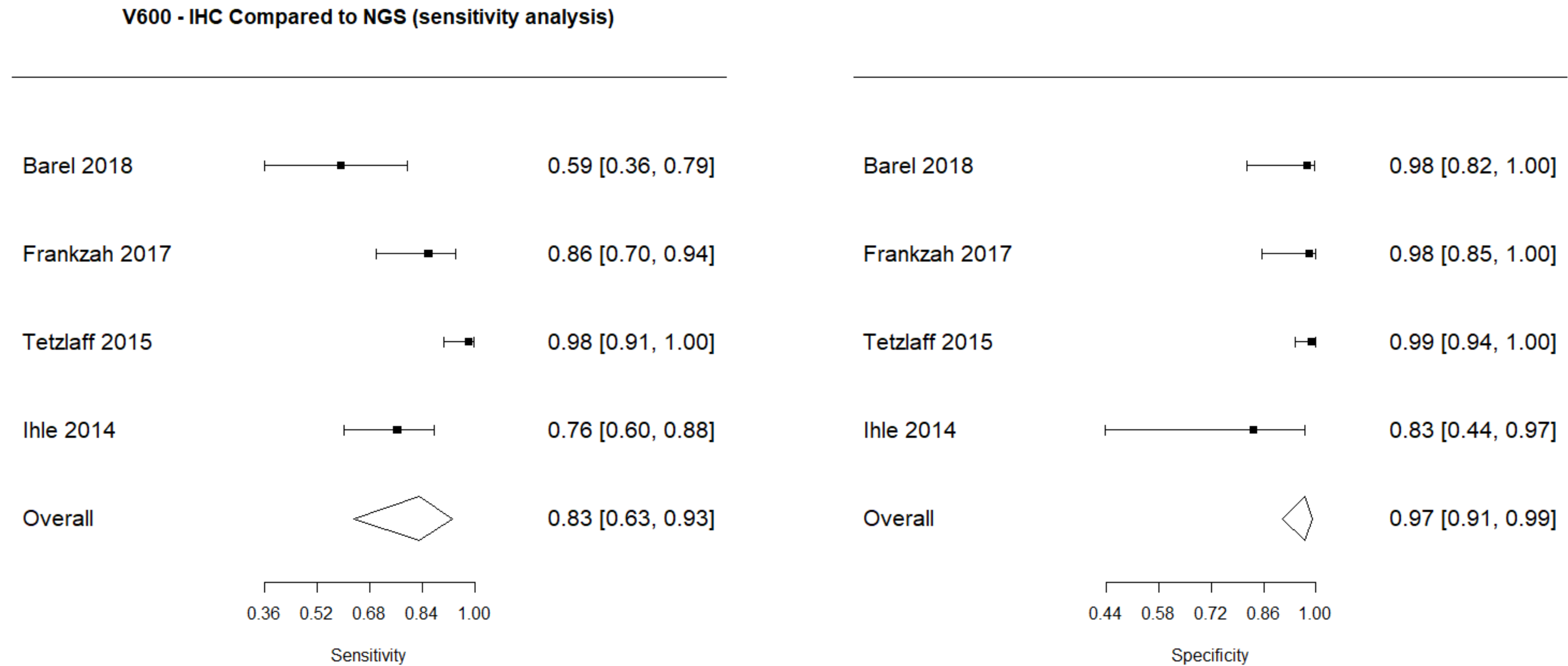
Sensitivity I^2= 67.3%, Specificity I^2= 23.8%

Figure 8: Likelihood ratios for IHC compared to NGS for the detection of any V600 mutation



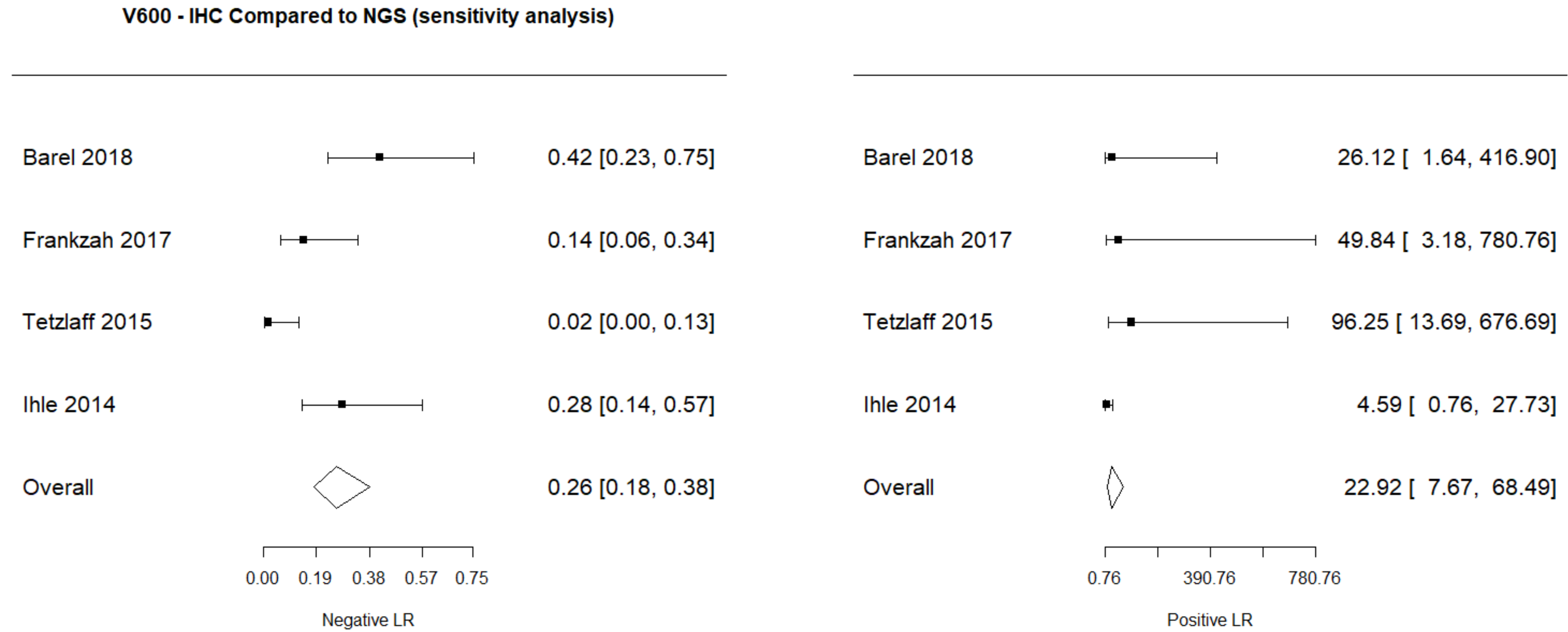
Positive likelihood ratio I^2= 38.0%, negative likelihood ratio I^2= 65.5%

Figure 9: Sensitivity and specificity for IHC compared to NGS for the detection of any V600 mutation, excluding high risk of bias studies



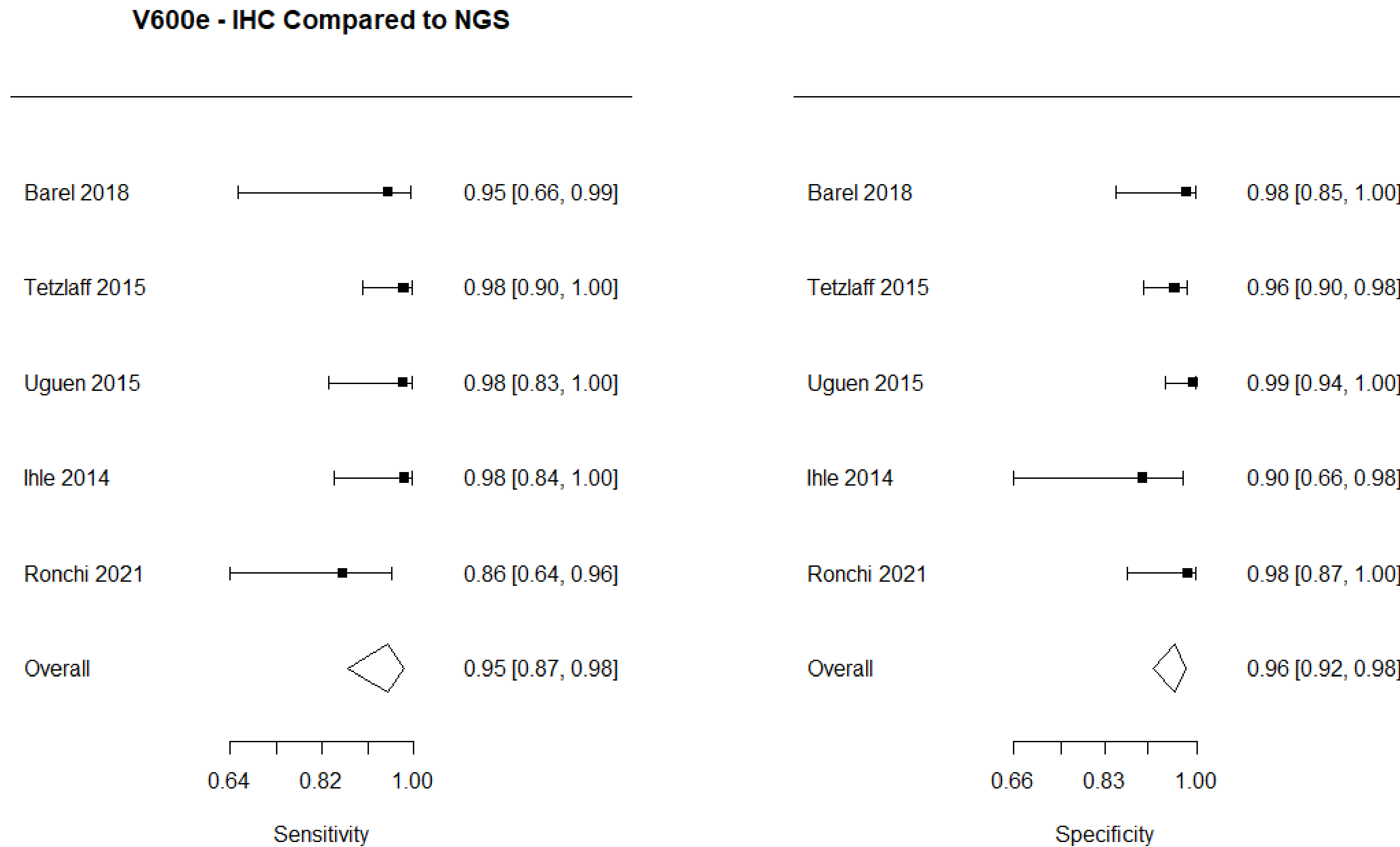
Sensitivity I^2= 74.0%, Specificity I^2= 30.2%

Figure 10: Likelihood ratios for IHC compared to NGS for the detection of any V600 mutation, excluding high risk of bias studies



Positive likelihood ratio I^2= 45.1%, negative likelihood ratio I^2= 74.0%

Figure 11: Sensitivity and specificity for IHC compared to NGS for the detection of V600e mutations

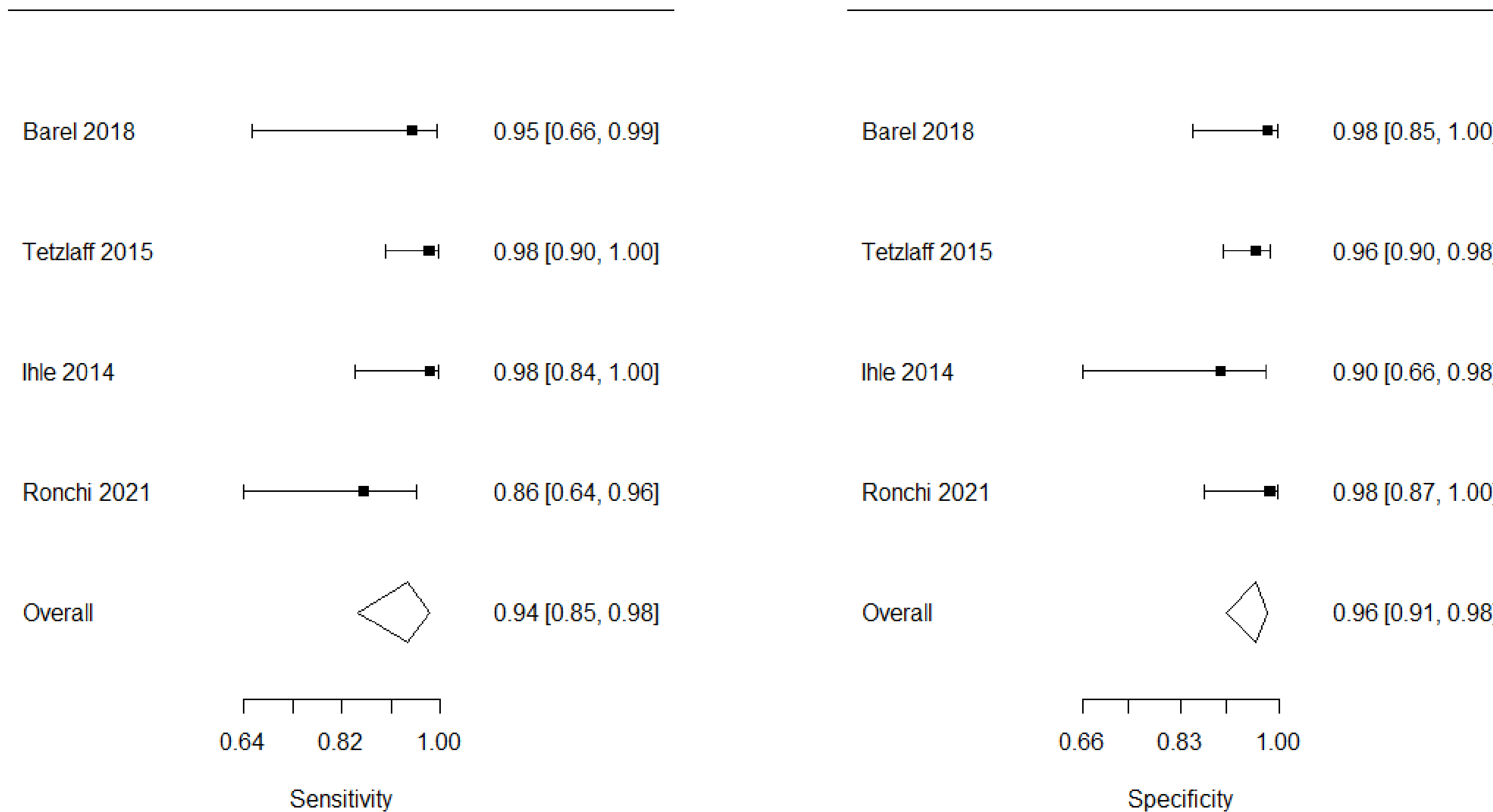


Sensitivity I^2= 0.0%, Specificity I^2= 12.7%

Figure 12: Likelihood ratios for IHC compared to NGS for the detection of V600e mutation

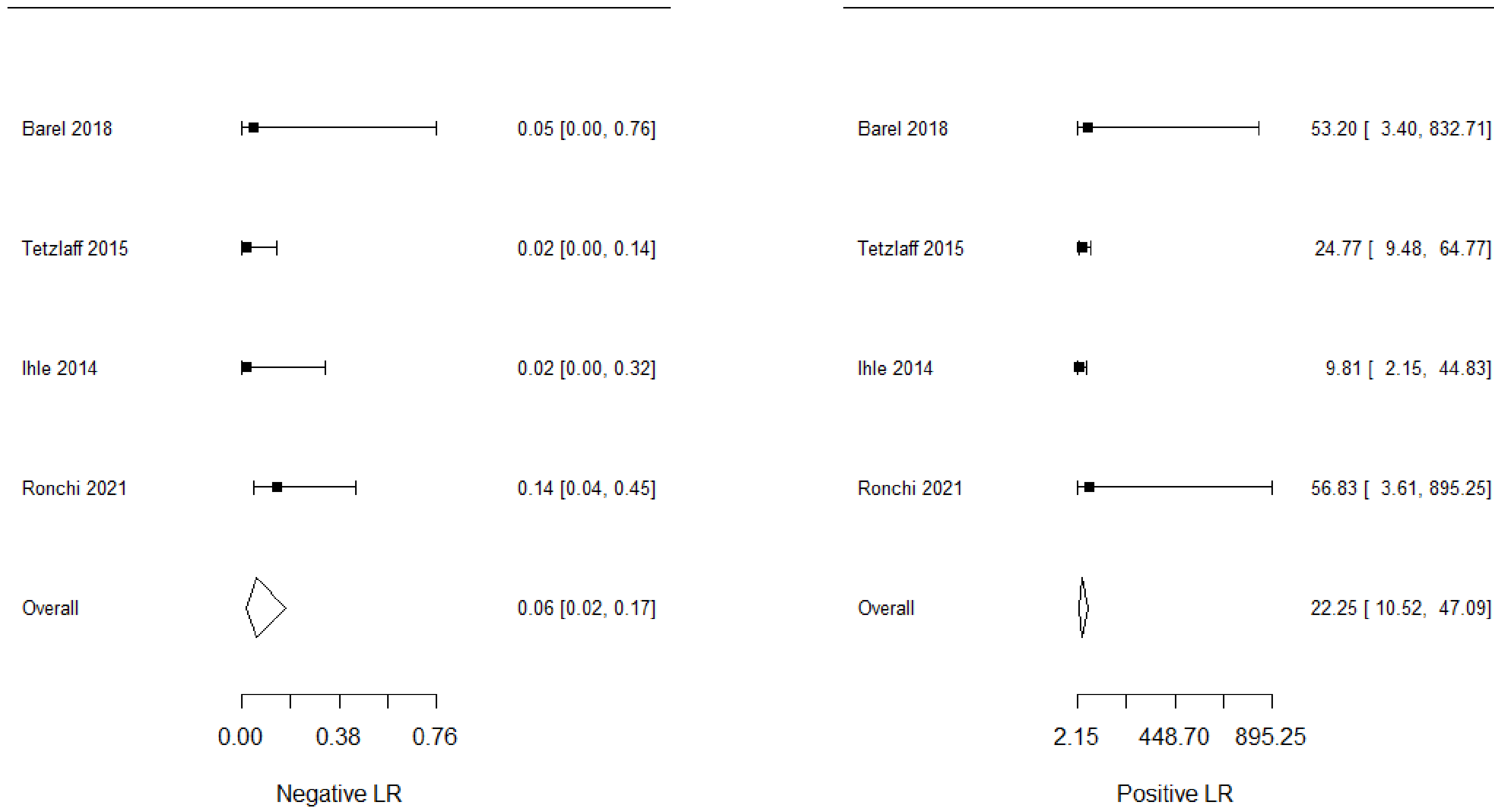
Positive likelihood ratio I^2= 13.1%, negative likelihood ratio I^2= 0.0%

Figure 13: Sensitivity and specificity for IHC compared to NGS for the detection of V600e mutations, excluding high risk of bias studies



Sensitivity I^2= 0.0%, Specificity I^2= 0.0%

Figure 14: Likelihood ratios for IHC compared to NGS for the detection of V600e mutations



Positive likelihood ratio I^2= 0.0%, negative likelihood ratio I^2= 0.0%

Appendix F – GRADE tables

Diagnostic accuracy of IHC using COBAS 4800 alone as a reference standard

No. of studies	Study design	Sample size	Sensitivity (95%CI)	Specificity (95%CI)	Prevalence of BRAF mutation	Likelihood ratios (95%CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	Quality
For the detection of any BRAF mutation (Figure 1 **and** Figure 2)											
9	Diagnostic accuracy	837	0.90 (0.86, 0.93)	0.92 (0.81, 0.97)	360 (43.0%)	LR+ 12.45 (4.62, 33.49)	Serious[1]	Not serious	Very serious[2]	Not serious	Very low
						LR- 0.11 (0.08, 0.15)	Serious[1]	Not serious	Not serious	Not serious	Moderate
For the detection of any BRAF mutation (sensitivity analysis excluding studies at high risk of bias) (Figure 3 **and** Figure 4**)**											
7	Diagnostic accuracy	686	0.91 (0.86, 0.94)	0.91 (0.76, 0.97)	301 (43.8%)	LR+ 10.41 (3.58, 30.32)	Serious[1]	Not serious	Very serious[2]	Not serious	Very low
						LR- 0.11 (0.07, 0.15)	Serious[1]	Not serious	Not serious	Not serious	Moderate

1. >33.3% of studies were at moderate or high risk of bias
2. I^2 >66.6%

Diagnostic accuracy of IHC using COBAS 4800 (with confirmation of discrepant cases) as a reference standard

No. of studies	Study design	Sample size	Sensitivity (95%CI)	Specificity (95%CI)	Prevalence of BRAF mutation	Likelihood ratios (95%CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	Quality
For the detection of any BRAF mutation (Figure 5 **and** Figure 6**)**											

No. of studies	Study design	Sample size	Sensitivity (95%CI)	Specificity (95%CI)	Prevalence of BRAF mutation	Likelihood ratios (95%CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	Quality
6	Diagnostic accuracy	745	0.89 (0.82, 0.94)	0.98 (0.96, 0.99)	330 (44.3%)	LR+ 55.49 (24.23, 127.06)	Serious[1]	Not serious	Not serious	Not serious	Moderate
						LR- 0.11 (0.06, 0.19)	Serious[1]	Not serious	Serious[2]	Not serious	Low

1. >33.3% of studies were at moderate or high risk of bias
2. I^2 >33.3%

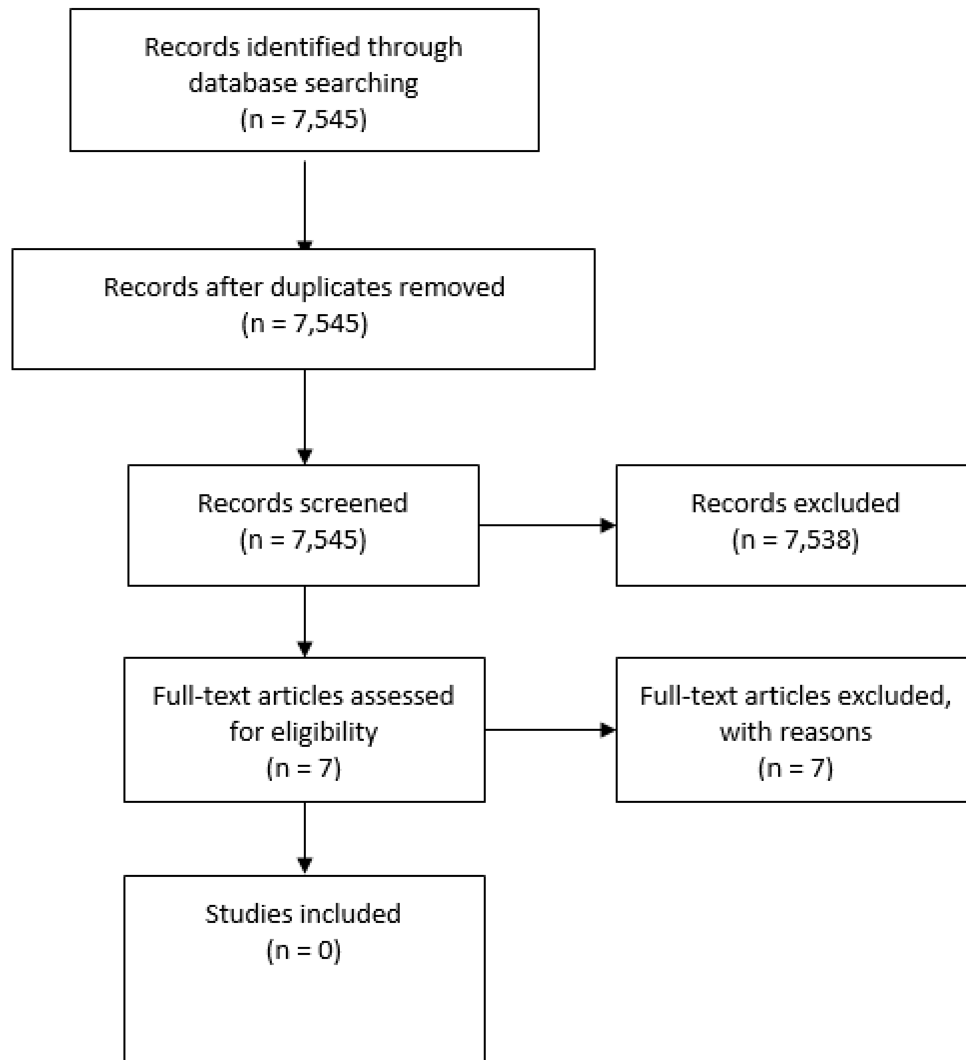
Diagnostic accuracy of IHC using NGS as a reference standard

No. of studies	Study design	Sample size	Sensitivity (95%CI)	Specificity (95%CI)	Prevalence of BRAF mutation	Likelihood ratios (95%CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	Quality
For the detection of any BRAF mutation (Figure 7 and Figure 8)											
5	Diagnostic accuracy	393	0.80 (0.65,0.90)	0.98 (0.93, 0.99)	169 (43.0%)	LR+ 28.10 (10.15, 77.79)	Serious[1]	Not serious	Serious[5]	Not serious	Low
						LR- 0.22 (0.12, 0.40)	Serious[1]	Not serious	Very serious[2]	Not serious	Very low
For the detection of any BRAF mutation (sensitivity analysis excluding studies at high risk of bias) (Figure 9 and Figure 10)											
4	Diagnostic accuracy	289	0.83 (0.63,0.93)	0.97 (0.91, 0.99)	136 (47.1%)	LR+ 22.92 (7.67, 68.49)	Not serious	Not serious	Serious[5]	Not serious	Moderate
						LR- 0.26 (0.18, 0.38)	Not serious	Not serious	Very serious[2]	Serious[4]	Very low
For the detection v600e mutations (Figure 11 and Figure 12)											

No. of studies	Study design	Sample size	Sensitivity (95%CI)	Specificity (95%CI)	Prevalence of BRAF mutation	Likelihood ratios (95%CI)	Risk of bias	Indirectness	Inconsistency	Imprecision	Quality
5	Diagnostic accuracy	383	0.95 (0.87,0.98)	0.96 (0.92, 0.98)	129 (33.7%)	LR+ 25.46 (12.35, 52.49)	Serious[1]	Not serious	Not serious	Not serious	Moderate
						LR- 0.06 (0.02, 0.14)	Serious[1]	Not serious	Not serious	Not serious	Moderate
For the detection v600e mutations (sensitivity analysis excluding studies at high risk of bias) (Figure 13 and Figure 14**)**											
4	Diagnostic accuracy	279	0.94 (0.85,0.98)	0.96 (0.91, 0.98)	125 (37.6%)	LR+ 22.25 (10.52, 47.09)	Serious[1]	Not serious	Not serious	Not serious	Moderate
						LR- 0.06 (0.02, 0.17)	Serious[1]	Not serious	Not serious	Not serious	Moderate

1. >33.3% of studies were at moderate or high risk of bias
2. I^2 >66.6%
3. 95% CIs cross one line of the MID (0.50)

Appendix G – Economic evidence study selection



Appendix H – Economic evidence tables

No economic evidence is available as none of the studies in the economic search results was found to be relevant.

Table 10 Economic Evidence Table for original model

Study	Study type	Setting	Interventions	Population	Methods of analysis	Base-case results	Sensitivity analyses	Additional comments
Original model	Cost effectiveness study Decision tree	UK Hospital National healthcare system	Genetic testing for BRAF mutations with Cobas alone versus IHC followed by Cobas if negative in patients with stage IIC or III melanoma	Patients with Stage IIC, III.	**Diagnostic accuracy:** Meta analysis of studies as reported in this review. **Costs:** Resource use extrapolated from Pasmans et al. (2019), Ryan et al. (2019) and committee input. Unit costs from NHS supply chain catalogue, Pasmans et al. (2019), and committee input.	For Stage IIC Costs: Cobas: £75,179 IHC & Cobas: £128,751 Effects: Cobas: 67.14 IHC & Cobas: 76.06 Incremental: Costs: £53,554 Effects: 9.91 For Stage III Costs: Cobas: £75,179 IHC & Cobas: £128,751 Effects: Cobas: 195.22 IHC & Cobas: 221.21 Incremental: Costs: £53,554 Effects: 26.01	**For stage IIC** **Deterministic:** Most sensitive to the cost of IHC testing. **Probabilistic:** Congruent to deterministic results. **For stage III** **Deterministic:** Most sensitive to the cost of IHC testing. **Probabilistic:** Congruent to deterministic results	Authors' conclusions: IHC & PCR Cobas results in a greater number of people appropriately receiving targeted therapy. However, this outcome is achieved at higher cost.

Appendix I – Excluded studies

Clinical studies

Study reference	Reason for exclusion
Anwar, Muhammad Ahmed Farooq, Murad, Fadi, Dawson, Erin et al. (2016) Immunohistochemistry as a reliable method for detection of BRAF-V600E mutation in melanoma: a systematic review and meta-analysis of current published literature. The Journal of surgical research 203(2): 407-15	- Systematic review used as source of primary studies
Arenberger, P, Arenbergerova, M, Vohradnikova, O et al. (2008) Early detection of melanoma progression by quantitative real-time RT-PCR analysis for multiple melanoma markers. The Keio journal of medicine 57(1): 57-64	- Did not look at relevant genes

Study reference	Reason for exclusion
Barbano, Raffaela, Pasculli, Barbara, Coco, Michelina et al. (2015) Competitive allele-specific TaqMan PCR (Cast-PCR) is a sensitive, specific and fast method for BRAF V600 mutation detection in Melanoma patients. Scientific reports 5: 18592	- Reference standard did not meet inclusion criteria
Boursault, Lucile, Haddad, Veronique, Vergier, Beatrice et al. (2013) Tumor homogeneity between primary and metastatic sites for BRAF status in metastatic melanoma determined by immunohistochemical and molecular testing. PloS one 8(8): e70826	- Reference standard did not meet inclusion criteria
Bruno, William, Martinuzzi, Claudia, Andreotti, Virginia et al. (2017) Heterogeneity and frequency of BRAF mutations in primary melanoma: Comparison between molecular methods and immunohistochemistry. Oncotarget 8(5): 8069-8082	- Could not create 2 x 2 table for relevant study population
Calbet-Llopart, N., Potrony, M., Tell-Marti, G. et al. (2020) Detection of cell-free circulating BRAFV 600E by droplet digital polymerase chain reaction in patients with and without melanoma under dermatological surveillance. British Journal of Dermatology 182(2): 382-389	- Outcome did not meet protocol
Chen, Qiongrong, Xia, Chunjiao, Deng, Yunte et al. (2014) Immunohistochemistry as a quick screening method for clinical detection of BRAF(V600E) mutation in melanoma patients. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 35(6): 5727-33	- Reference standard did not meet inclusion criteria
Chen, Tai-Long, Chang, John Wen-Cheng, Hsieh, Jia-Juan et al. (2016) A Sensitive Peptide Nucleic Acid Probe Assay for Detection of BRAF V600 Mutations in Melanoma. Cancer genomics & proteomics 13(5): 381-6	- Did not look at immunohistochemistry
Cheng, Liang, Lopez-Beltran, Antonio, Massari, Francesco et al. (2018) Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 31(1): 24-38	- Did not look at immunohistochemistry
Colomba E, Hélias-Rodzewicz Z, Von Deimling A et al. (2013) Detection of BRAF p.V600E mutations in melanomas: comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing. The Journal of molecular diagnostics : JMD 15(1): 94-100	- Reference standard did not meet inclusion criteria
Colombino, Maria, Rozzo, Carla, Paliogiannis, Panagiotis et al. (2020) Comparison of BRAF Mutation Screening Strategies in a Large Real-Life Series of Advanced Melanoma Patients. Journal of clinical medicine 9(8)	- Did not look at immunohistochemistry
Corean, J.L.E., George, T.I., Patel, J.L. et al. (2019) Bone marrow findings in metastatic melanoma, including role of BRAF immunohistochemistry. International Journal of Laboratory Hematology 41(4): 550-560	- Reference standard did not meet inclusion criteria
Emile, Jean-Francois, Tisserand, Julie, Bergougnoux, Loic et al. (2013) Improvement of the quality of BRAF testing in melanomas with nationwide external quality assessment, for the BRAF EQA group. BMC cancer 13: 472	- Did not look at immunohistochemistry
Eriksson, H., Zebary, A., Vassilaki, I., Omholt, K., Ghaderi, M., & Hansson, J. (2015). BRAFV600E protein expression in primary cutaneous malignant melanomas and paired metastases. JAMA dermatology, 151(4), 410-416.	- Reference standard did not meet inclusion criteria
Etienne, M., Oca, F., Prunier-Mirebeau, D. et al. (2018) Immunohistochemistry using clone VE1 is an economic,	- Non-English language paper

Study reference	Reason for exclusion
specific and sensitive method for detecting the presence of BRAFV600E mutations in melanoma. Annales de Dermatologie et de Venereologie 145(3): 159-165	
Fatnassi-Mersni, G., Arfaoui, A.T., Cherni, M. et al. (2020) Molecular and Immunohistochemical Analysis of BRAF gene in Primary Cutaneous Melanoma: Discovery of novel mutations. Journal of cutaneous pathology	- Reference standard did not meet inclusion criteria
Garg, S., Grenier, S., Misyura, M. et al. (2020) Assessing the Diagnostic Yield of Targeted Next-Generation Sequencing for Melanoma and Gastrointestinal Tumors. Journal of Molecular Diagnostics 22(4): 467-475	- Did not look at immunohistochemistry
Harle, Alexandre, Salleron, Julia, Franczak, Claire et al. (2016) Detection of BRAF Mutations Using a Fully Automated Platform and Comparison with High Resolution Melting, Real-Time Allele Specific Amplification, Immunohistochemistry and Next Generation Sequencing Assays, for Patients with Metastatic Melanoma. PloS one 11(4): e0153576	- Same sample used in another included study
How-Kit, Alexandre, Lebbe, Celeste, Bousard, Aurelie et al. (2014) Ultrasensitive detection and identification of BRAF V600 mutations in fresh frozen, FFPE, and plasma samples of melanoma patients by E-ice-COLD-PCR. Analytical and bioanalytical chemistry 406(22): 5513-20	- Did not look at immunohistochemistry
Huang, Wen-Kuan, Kuo, Tseng-Tong, Wu, Chiao-En et al. (2016) A comparison of immunohistochemical and molecular methods used for analyzing the BRAF V600E gene mutation in malignant melanoma in Taiwan. Asia-Pacific journal of clinical oncology 12(4): 403-408	- Reference standard did not meet inclusion criteria
Ihle, Michaela Angelika, Fassunke, Jana, Konig, Katharina et al. (2014) Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p.V600E and non-p.V600E BRAF mutations. BMC cancer 14: 13	- Could not separate out melanoma population from overall cohort
Jabbar KJ, Luthra R, Patel KP et al. (2015) Comparison of next-generation sequencing mutation profiling with BRAF and IDH1 mutation-specific immunohistochemistry. The American journal of surgical pathology 39(4): 454-461	- Could not separate out melanoma population from overall cohort
Jurkowska, Monika, Gos, Aleksandra, Ptaszynski, Konrad et al. (2015) Comparison between two widely used laboratory methods in BRAF V600 mutation detection in a large cohort of clinical samples of cutaneous melanoma metastases to the lymph nodes. International journal of clinical and experimental pathology 8(7): 8487-93	- Did not look at immunohistochemistry
Just PA, Audebourg A, Pasmant E et al. (2014) Immunohistochemistry versus next-generation sequencing for the routine detection of BRAF V600E mutation in melanomas. Human pathology 45(9): 1983-1984	- Letter to editor
Kakavand, Hojabr, Walker, Emily, Lum, Trina et al. (2016) BRAF(V600E) and NRAS(Q61L/Q61R) mutation analysis in metastatic melanoma using immunohistochemistry: a study of 754 cases highlighting potential pitfalls and guidelines for interpretation and reporting. Histopathology 69(4): 680-6	- Reference standard did not meet inclusion criteria
Knol, A..-C., Pandolfino, M..-C., Vallee, A. et al. (2015) Comparative analysis of BRAF, NRAS and c-KIT mutation status between tumor tissues and autologous tumor cell-lines	- Reference standard did not meet inclusion criteria

Study reference	Reason for exclusion
of stage III/IV melanoma. Experimental Dermatology 24(1): 70-73	
Lamy, Pierre-Jean, Castan, Florence, Lozano, Nicolas et al. (2015) Next-Generation Genotyping by Digital PCR to Detect and Quantify the BRAF V600E Mutation in Melanoma Biopsies. The Journal of molecular diagnostics : JMD 17(4): 366-73	- Reference standard did not meet inclusion criteria
Leblond, Anne-Laure, Rechsteiner, Markus, Jones, Amy et al. (2019) Microfluidic-Based Immunohistochemistry Combined With Next-Generation Sequencing on Diagnostic Tissue Sections for Detection of Tumoral BRAF V600E Mutation. American journal of clinical pathology 152(1): 59-73	- Could not separate out melanoma population from overall cohort
Loes, Inger Marie, Immervoll, Heike, Angelsen, Jon-Helge et al. (2015) Performance comparison of three BRAF V600E detection methods in malignant melanoma and colorectal cancer specimens. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 36(2): 1003-13	- Reference standard did not meet inclusion criteria
Long, E, Ilie, M, Lassalle, S et al. (2015) Why and how immunohistochemistry should now be used to screen for the BRAFV600E status in metastatic melanoma? The experience of a single institution (LCEP, Nice, France). Journal of the European Academy of Dermatology and Venereology : JEADV 29(12): 2436-43	- Reference standard did not meet inclusion criteria
Loo, Eric, Khalili, Parisa, Beuhler, Karen et al. (2018) BRAF V600E Mutation Across Multiple Tumor Types: Correlation Between DNA-based Sequencing and Mutation-specific Immunohistochemistry. Applied immunohistochemistry & molecular morphology : AIMM 26(10): 709-713	- Reference standard did not meet inclusion criteria
Liu, Hui, Li, Zhongwu, Wang, Yan et al. (2014) Immunohistochemical detection of the BRAF V600E mutation in melanoma patients with monoclonal antibody VE1. Pathology international 64(12): 601-6	- Reference standard did not meet inclusion criteria
Malicherova, B., Burjanivova, T., Grendar, M. et al. (2018) Droplet digital PCR for detection of BRAF V600E mutation in formalin-fixed, paraffin-embedded melanoma tissues: A comparison with Cobas 4800, sanger sequencing, and allele-specific PCR. American Journal of Translational Research 10(11): 3773-3781	- Could not create 2 x 2 table for relevant study population
Mancini, I., Simi, L., Salvianti, F. et al. (2019) Analytical evaluation of an NGS testing method for routine molecular diagnostics on melanoma formalin-fixed, paraffin-embedded tumor-derived DNA. Diagnostics 9(3): 117	- Did not look at immunohistochemistry
Manfredi, Laure, Meyer, Nicolas, Tournier, Emilie et al. (2016) Highly Concordant Results Between Immunohistochemistry and Molecular Testing of Mutated V600E BRAF in Primary and Metastatic Melanoma. Acta dermato-venereologica 96(5): 630-4	- Reference standard did not meet inclusion criteria
Marchant, Julie, Mange, Alain, Larrieux, Marion et al. (2014) Comparative evaluation of the new FDA approved THxID TM-BRAF test with High Resolution Melting and Sanger sequencing. BMC cancer 14: 519	- Did not look at immunohistochemistry
Marin, Cristi, Beauchet, Alain, Capper, David et al. (2014) Detection of BRAF p.V600E Mutations in Melanoma by Immunohistochemistry Has a Good Interobserver	- Reference standard did not meet inclusion criteria

Study reference	Reason for exclusion
Reproducibility. Archives of pathology & laboratory medicine 138(1): 71-5	
Massi, Daniela, Simi, Lisa, Sensi, Elisa et al. (2015) Immunohistochemistry is highly sensitive and specific for the detection of NRASQ61R mutation in melanoma. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 28(4): 487-97	- Could not create 2 x 2 table for relevant study population
McEvoy, Ashleigh C, Wood, Benjamin A, Ardakani, Nima M et al. (2018) Droplet Digital PCR for Mutation Detection in Formalin-Fixed, Paraffin-Embedded Melanoma Tissues: A Comparison with Sanger Sequencing and Pyrosequencing. The Journal of molecular diagnostics : JMD 20(2): 240-252	- Did not look at immunohistochemistry
Melchior, Linea, Grauslund, Morten, Bellosillo, Beatriz et al. (2015) Multi-center evaluation of the novel fully-automated PCR-based Idylla TM BRAF Mutation Test on formalin-fixed paraffin-embedded tissue of malignant melanoma. Experimental and molecular pathology 99(3): 485-91	- Reference standard did not meet inclusion criteria
Mourah, Samia, Denis, Marc G, Narducci, Fabienne Escande et al. (2015) Detection of BRAF V600 mutations in melanoma: evaluation of concordance between the Cobas R 4800 BRAF V600 mutation test and the methods used in French National Cancer Institute (INCa) platforms in a real-life setting. PloS one 10(3): e0120232	- Did not look at immunohistochemistry
Orchard, G E, Wojcik, K, Rickaby, W et al. (2019) Immunohistochemical detection of V600E BRAF mutation is a useful primary screening tool for malignant melanoma. British journal of biomedical science 76(2): 77-82	- Reference standard did not meet inclusion criteria
Panka, David J, Buchbinder, Elizabeth, Giobbie-Hurder, Anita et al. (2014) Clinical utility of a blood-based BRAF(V600E) mutation assay in melanoma. Molecular cancer therapeutics 13(12): 3210-8	- Did not look at immunohistochemistry
Pearlstein, Michelle V, Zedek, Daniel C, Ollila, David W et al. (2014) Validation of the VE1 immunostain for the BRAF V600E mutation in melanoma. Journal of cutaneous pathology 41(9): 724-32	- Reference standard did not meet inclusion criteria
Pellegrini, Cristina, Di Nardo, Lucia, Cipolloni, Gianluca et al. (2018) Heterogeneity of BRAF, NRAS, and TERT Promoter Mutational Status in Multiple Melanomas and Association with MC1R Genotype: Findings from Molecular and Immunohistochemical Analysis. The Journal of molecular diagnostics : JMD 20(1): 110-122	- Reference standard did not meet inclusion criteria
Petty, D.R., Hassan, O.A., Barker, C.S. et al. (2020) Rapid BRAF Mutation Testing in Pigmented Melanomas. The American Journal of dermatopathology 42(5): 343-348	- Did not look at immunohistochemistry
Pisareva, Ekaterina, Gutkina, Nadezhda, Kovalenko, Sergei et al. (2014) Sensitive allele-specific real-time PCR test for mutations in BRAF codon V600 in skin melanoma. Melanoma research 24(4): 322-31	- Did not look at immunohistochemistry
Ponti, Giovanni, Tomasi, Aldo, Maiorana, Antonio et al. (2016) BRAFp.V600E, p.V600K, and p.V600R Mutations in Malignant Melanoma: Do They Also Differ in Immunohistochemical Assessment and Clinical Features?. Applied immunohistochemistry & molecular morphology : AIMM 24(1): 30-4	- Reference standard did not meet inclusion criteria

Study reference	Reason for exclusion
Qiu, T., Lu, H., Guo, L., Huang, W., Ling, Y., Shan, L., ... & Lv, N. (2015). Detection of BRAF mutation in Chinese tumor patients using a highly sensitive antibody immunohistochemistry assay. Scientific reports, 5, 9211.	- Reference standard did not meet inclusion criteria
Qu, Kevin, Pan, Qiulu, Zhang, Xi et al. (2013) Detection of BRAF V600 mutations in metastatic melanoma: comparison of the Cobas 4800 and Sanger sequencing assays. The Journal of molecular diagnostics : JMD 15(6): 790-5	- Did not look at immunohistochemistry
Reid, Anna L, Freeman, James B, Millward, Michael et al. (2015) Detection of BRAF-V600E and V600K in melanoma circulating tumour cells by droplet digital PCR. Clinical biochemistry 48(15): 999-1002	- Did not look at immunohistochemistry
Richter, Anna, Grieu, Fabienne, Carrello, Amerigo et al. (2013) A multisite blinded study for the detection of BRAF mutations in formalin-fixed, paraffin-embedded malignant melanoma. Scientific reports 3: 1659	- Did not look at immunohistochemistry
Routhier, Caitlin Ann, Mochel, Mark C, Lynch, Kerry et al. (2013) Comparison of 2 monoclonal antibodies for immunohistochemical detection of BRAF V600E mutation in malignant melanoma, pulmonary carcinoma, gastrointestinal carcinoma, thyroid carcinoma, and gliomas. Human pathology 44(11): 2563-70	- Reference standard did not meet inclusion criteria
Salvianti, Francesca, Massi, Daniela, De Giorgi, Vincenzo et al. (2019) Evaluation of the liquid biopsy for the detection of BRAFV600E mutation in metastatic melanoma patients. Cancer biomarkers : section A of Disease markers 26(3): 271-279	- Did not look at immunohistochemistry
Schafroth, Christian, Galvan, Jose A, Centeno, Irene et al. (2015) VE1 immunohistochemistry predicts BRAF V600E mutation status and clinical outcome in colorectal cancer. Oncotarget 6(39): 41453-63	- Could not separate out melanoma population from overall cohort
Schiefer, Ana-Iris, Parlow, Laura, Gabler, Lisa et al. (2016) Multicenter Evaluation of a Novel Automated Rapid Detection System of BRAF Status in Formalin-Fixed, Paraffin-Embedded Tissues. The Journal of molecular diagnostics : JMD 18(3): 370-377	- Did not look at immunohistochemistry
Serre, D., Salleron, J., Husson, M. et al. (2018) Accelerated BRAF mutation analysis using a fully automated PCR platform improves the management of patients with metastatic melanoma. Oncotarget 9(63): 32232-32237	- Did not look at immunohistochemistry
Seto, K., Haneda, M., Masago, K. et al. (2020) Negative reactions of BRAF mutation-specific immunohistochemistry to non-V600E mutations of BRAF. Pathology International 70(5): 253-261	- Included all melanomas without information on tumour stage or timing of tests
Shapochka, D, Shapochka, T, Seleznyov, O et al. (2018) USE OF CIRCULATING TUMOR DNA FOR DETECTION OF BRAF V600E MUTATION AND TREATMENT MONITORING IN MELANOMA PATIENTS. Georgian medical news: 76-81	- Did not look at immunohistochemistry
Shofty, B., Artzi, M., Shtrozberg, S. et al. (2020) Virtual biopsy using MRI radiomics for prediction of BRAF status in melanoma brain metastasis. Scientific reports 10(1): 6623	- Did not look at immunohistochemistry
Skorokhod, Alexander (2015) Universal BRAF State Detection by the Pyrosequencing R-Based U-BRAF(V600) Assay. Methods in molecular biology (Clifton, N.J.) 1315: 63-82	- Did not look at immunohistochemistry

Study reference	Reason for exclusion
Thiel, Alexandra, Moza, Monica, Kytola, Soili et al. (2015) Prospective immunohistochemical analysis of BRAF V600E mutation in melanoma. Human pathology 46(2): 169-75	- Reference standard did not meet inclusion criteria
Tzanikou, E., Haselmann, V., Markou, A. et al. (2020) Direct comparison study between droplet digital PCR and a combination of allele-specific PCR, asymmetric rapid PCR and melting curve analysis for the detection of BRAF V600E mutation in plasma from melanoma patients. Clinical Chemistry and Laboratory Medicine	- Could not create 2 x 2 table for relevant study population
Uguen, Arnaud, Talagas, Matthieu, Costa, Sebastian et al. (2015) NRAS (Q61R), BRAF (V600E) immunohistochemistry: a concomitant tool for mutation screening in melanomas. Diagnostic pathology 10: 121	- Reference standard did not meet inclusion criteria
Vallee, Audrey, Denis-Musquer, Marie, Herbreteau, Guillaume et al. (2019) Prospective evaluation of two screening methods for molecular testing of metastatic melanoma: Diagnostic performance of BRAF V600E immunohistochemistry and of a NRAS-BRAF fully automated real-time PCR-based assay. PloS one 14(8): e0221123	- Reference standard did not meet inclusion criteria
Van Haele, Matthias, Vander Borght, Sara, Ceulemans, An et al. (2020) Rapid clinical mutational testing of KRAS, BRAF and EGFR: a prospective comparative analysis of the Idylla technique with high-throughput next-generation sequencing. Journal of clinical pathology 73(1): 35-41	- Did not look at immunohistochemistry
Yaman, Banu; Kandiloglu, Gulsen; Akalin, Taner (2016) BRAF-V600 Mutation Heterogeneity in Primary and Metastatic Melanoma: A Study With Pyrosequencing and Immunohistochemistry. The American Journal of dermatopathology 38(2): 113-20	- Reference standard did not meet inclusion criteria
Zhang, W., Song, G., Han, X. et al. (2017) A validation study for the use VE1 immunohistochemical staining in screening for BRAF mutation in cutaneous malignant melanoma. Biomedical Research (India) 28(11): 4886-4890	- Reference standard did not meet inclusion criteria
Zhu, Meng-Lei; Zhou, Lan; Sadri, Navid (2018) Comparison of targeted next generation sequencing (NGS) versus isolated BRAF V600E analysis in patients with metastatic melanoma. Virchows Archiv : an international journal of pathology 473(3): 371-377	- Did not look at immunohistochemistry

Economic studies

Study reference	Reason for exclusion
Kansal AR, Shaul AJ, Stern S, Busam K, Doucet CA, Chalfin DB. Cost–effectiveness of a FISH assay for the diagnosis of melanoma in the USA. Expert review of pharmacoeconomics & outcomes research. 2013 Jun 1;13(3):371-80.	- Study does not contain a relevant intervention [Compares use of FISH staining versus no staining]
Li Y, Bare LA, Bender RA, Sninsky JJ, Wilson LS, Devlin JJ, Waldman FM. Cost effectiveness of sequencing 34 cancer-associated genes as an aid for treatment selection in patients with metastatic melanoma. Molecular diagnosis & therapy. 2015 Jun 1;19(3):169-77.	- Study is not in a representative setting [USA]
Pasmans, Clemence T B, Tops, Bastiaan B J, Steeghs, Elisabeth M P et al. (2021) Micro-costing diagnostics in oncology: from single-gene testing to whole- genome	- Microcosting analysis, did not include outcomes

Study reference	Reason for exclusion
sequencing. Expert review of pharmacoeconomics & outcomes research 21(3): 413-414	
Ronchi, Andrea, Montella, Marco, Marino, Federica Zito et al. (2021) Predictive evaluation on cytological sample of metastatic melanoma: The role of braf immunocytochemistry in the molecular era. Diagnostics 11(6): 1110	- Study did not include an economic evaluation
Seo MK, Straume O, Akslen LA, Cairns J. HSP27 Expression as a Novel Predictive Biomarker for Bevacizumab: is it Cost Effective?. PharmacoEconomics-Open. 2020 Jan 27:1-1.	- Target mutation was not BRAF status
Van Amerongen RA, Retèl VP, Coupé VM, Nederlof PM, Vogel MJ, Van Harten WH. Next-generation sequencing in NSCLC and melanoma patients: a cost and budget impact analysis. ecancermedicalscience. 2016;10.	- Study did not include outcomes
Wu B, Shi L. Frontline BRAF Testing–Guided Treatment for Advanced Melanoma in the Era of Immunotherapies: A Cost-Utility Analysis Based on Long-term Survival Data. JAMA dermatology. 2020 Jul 22.	- Study is not in a representative setting [USA]

Appendix J – Research recommendations – full details

Research recommendation 1 (Monitoring and response biomarkers: Biomarkers in place of imaging)

1. Can biomarkers accurately classify recurrence, progression and response to treatment?

Why this is important?

Biomarkers are of increasing relevance in the diagnosis and monitoring of various cancers however their utility in the context of the follow-up of melanoma is still unclear. There is a need to understand whether biomarkers can be used in place of imaging to detect disease recurrence and/or disease progression, and whether biomarkers can accurately assess whether a person has responded to treatment.

Rationale for research recommendation 1

Importance to 'patients' or the population	Use of biomarkers after beginning treatment has a number of potential uses. If biomarkers can accurately detect recurrence/progression, then people with melanoma may be able to safely reduce imaging requirements, which are costly, time consuming and can cause anxiety.
Relevance to NICE guidance	2021 update of the melanoma guideline recommends considering cross sectional and ultrasound imaging for stages IIB-C. However, there is very limited evidence for this use. Evidence for the effectiveness of biomarkers in the detection of recurrence may allow the complete or partial replacement of imaging with biomarker analysis for certain groups of people with melanoma.
Relevance to the NHS	Biomarker surveillance represents a quick, cheaper alternative to cross sectional imaging.
National priorities	High
Current evidence base	Very limited data specifically to melanoma
Equality considerations	None known

Modified PICO table

Population	• People receiving follow-up after stage IIB-IV melanoma • People receiving treatment for stage IIB-IV melanoma
Intervention (index test)	Biomarker analysis at the following timepoints: • When recurrence is suspected • During routine follow-up • After having receiving treatment for a predefined period of time
Comparator (reference standard)	Standard care (combination of physical exam and imaging)
Outcome	Detection of recurrence, assessed by sensitivity/specificity
Study design	Diagnostic accuracy study
Timeframe	Short term
Additional information	None

Research recommendation 2 (Safety, prognostic and predictive biomarkers to aid treatment stratification and selection: Biomarkers checked prior to treatment)

2. Can biomarkers be used for risk stratification and treatment planning for people with melanoma?

Why this is important

There is a need to understand whether biomarkers can be used in the pre-treatment period for risk stratification and to identify optimal treatment options, particularly in the following contexts:

- Can biomarkers be used for risk stratification and treatment selection within the substages?
- Can biomarkers be used to predict response to different treatments?
- Can biomarkers be used to predict treatment toxicity?
- Can biomarkers be used to assess extent of disease and predict response to surgery (such as whether melanoma is successfully completely excised)?

Rationale for research recommendation 2

Importance to 'patients' or the population	Biomarkers have the potential to be used for treatment evaluation and prognosis. This would allow for more personalised treatment regimens and allow for quicker adjustments to treatment.
Relevance to NICE guidance	NICE guidance is lacking recommendations on the use of biomarkers to guide treatment options. This research would allow for biomarker analysis at the time surrounding diagnosis to guide subsequent decisions.
Relevance to the NHS	Biomarkers represent an opportunity for more individualised medicine, better risk-stratification, and the identification of optimal treatments.
National priorities	High
Current evidence base	Very limited data specific to melanoma
Equality considerations	None known

Modified PICO table

Population	People with a diagnosis of melanoma
Intervention	Biomarker analysis
Comparator	None
Outcome	• Complete treatment response • Treatment-related adverse events and all serious adverse events • All-cause mortality • Disease progression/recurrence • Outcome following surgery (was surgery successful, was cancer completely excised?). Subgroup analyses will be conducted for treatment received
Study design	Prospective cohort study
Timeframe	Long term