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A Systematic Review and Meta-Analysis of the Diagnostic Performance of BRAF V600E Immunohistochemistry in Thyroid Histopathology

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Abstract

Immunohistochemistry (IHC) in evaluating thyroid surgical specimens may facilitate diagnostic and prognostic evaluation, with potential therapeutic implications. We performed a systematic review and meta-analysis examining the analytic validity of IHC in detecting *BRAFV600E* mutations in thyroid cancer (primary or metastatic). We screened citations from three electronic databases (until December 20, 2018), supplemented by a hand search of authors' files and cross-references of reviews. Citations and full-text papers were independently reviewed in duplicate, and consensus was achieved on inclusion of papers. Two reviewers independently critically appraised and abstracted data from included papers. Random-effect meta-analyses were conducted for sensitivity and specificity estimates. We reviewed 1499 unique citations and 93 full-text articles. We included 1 systematic review and 30 original articles. The published review (from 2015) needed to be updated as there were multiple subsequent original studies. The pooled sensitivity of IHC in detecting a *BRAFV600E* mutation was 96.8% (95% confidence interval [CI] at 94.1%, 98.3%) (29 studies, including 2659 *BRAFV600E* mutant tumors). The IHC pooled specificity was 86.3% (95% CI 80.7%, 90.4%) (28 studies, including 1107 *BRAFV600E* wild-type specimens). These meta-analyses were subject to statistically significant heterogeneity, partly explained by antibody type (sensitivity and specificity) and tissue/tumor type (specificity). In conclusion, BRAF IHC is highly sensitive and reasonably specific in detecting the *BRAFV600E* mutation; however, there is some variability in analytic performance.

Keywords *BRAFV600E* · Thyroid cancer · Diagnostic accuracy · Systematic review · Meta-analysis · Papillary thyroid carcinoma

Introduction

The incidence rate of thyroid cancer is rising, and the most common subtype is papillary thyroid cancer (PTC) [1]. The molecular pathogenesis of follicular epithelial-derived thyroid neoplasms has evolved the classification of PTCs, reflecting genotype–phenotype correlations of BRAF-like, RAS-like, and rare neutral (non-BRAF/RAS) phenotypes [2]. Among these, BRAF-like tumors represent mainly PTCs with classical architecture and/or solid growth; these tumors are enriched in molecular alterations including but not limited to *BRAFV600E* mutations (most common) and *RET/PTC* and *BRAF* fusions [2]. It has been reported that the prevalence of *BRAF* mutation in PTCs is increasing over time [3]. In

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contrast, RAS-like tumors encompass a spectrum of proliferations displaying exclusive follicular growth that includes lesions classified as follicular adenoma, non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) (formerly known as non-invasive encapsulated/well-demarcated follicular variant PTC), follicular variant PTC, and follicular thyroid carcinoma [4]. These tumors are enriched in *RAS* mutations; however, other molecular alterations, including *BRAFK601E* mutations and oncogene fusions (e.g., *PPARG*, *THADA*, and *FGFR2* fusions) also contribute to this phenotype [2]. Progress in the understanding of molecular oncology is reflected in the 2017 WHO classification of thyroid cancers [4].

Despite the rate of progress in the understanding of molecular biology of thyroid cancer, molecular testing of thyroid histopathology specimens is not universally accessible. Therefore, the rationale for using immunohistochemistry (IHC) in detecting *BRAFV600E* mutations is that IHC may be more time efficient, less expensive, and more easily accessible than molecular testing [5]. It may allow characterization of small lesions and distinction of molecular profiles in multiple lesions by assessing their morphology, unlike molecular testing that may cross-contaminate lesions. This study aimed to determine the sensitivity and specificity of IHC in detecting *BRAFV600E* mutations, as ascertained by gold standard molecular testing.

Methods

Research Objectives and Study Eligibility Criteria

Our primary research objective was to determine the sensitivity and specificity of immunohistochemistry for the *BRAFV600E* mutation in thyroid histopathology specimens in a systematic review of the published literature. Subgroup analyses were planned according to histologic diagnosis (i.e., type of thyroid cancer, benign thyroid tissue, ectopic thyroid tissue). This systematic review was registered (PROSPERO 2018 CRD42018086821). Although our primary focus was thyroid cancer, benign neoplasms, ectopic thyroid tissue, and other benign thyroid tissue were included as *BRAFV600E* wild-type controls. We only included studies in English (due to a lack of resources for translation). Prospective and retrospective published cross-sectional, cohort, case series, and case control studies were eligible if they reported data on sensitivity or specificity (or reported sufficient detail to calculate these values) of BRAFV600E IHC compared to molecular gold standard tests (e.g., direct sequencing, RT-PCR, or other DNA- or RNA-based molecular method). Published systematic reviews were also reviewed. Randomized trials were not relevant to our question.

Search for Relevant Studies and Study Selection

An experienced librarian (LP) searched Ovid MEDLINE, Ovid Medline In-Process & Other Non-Indexed Citations, Ovid Medline E-pub Ahead of Print, Ovid EMBASE, the Cochrane Central Register of Controlled Trials (Wiley), and the Cochrane Database of Systematic Reviews (Wiley) on January 10, 2018, and re-ran the search on December 20, 2018 (restricted to the years 2005 and later [given the time frame of development of the diagnostic of interest], with no language restrictions). Appropriate wildcard search terms were used in order to account for plurals and variations in spelling, relating to the terms BRAFV600E, thyroid, and immunohistochemistry. Clinicaltrials.gov was searched for the combination of BRAF and thyroid cancer on February 12, 2019 (by AMS). We also reviewed relevant references suggested by co-authors (AMS, OM). Reference lists of any reviews and included papers were scanned. Two reviewers (RS, AMS) independently screened the citations and abstracts retrieved from the electronic search for relevance, and any citation or abstract deemed relevant by either reviewer was independently reviewed in full-text form by both reviewers. Consensus was achieved by both reviewers for inclusion of papers, and a third reviewer (endocrine pathologist, OM) was consulted in the case of any disagreements or questions.

Critical Appraisal of Included Studies and Data Abstraction

Two reviewers (RS, AMS) independently evaluated study quality using QUADAS-2 [6] for original studies. QUADAS-2 evaluates the risk of bias in 4 domains (patient selection [e.g., random, consecutive], index test conduct and interpretation [e.g., blinding to index test, pre-set positivity threshold], reference test conduct and interpretation [e.g., likelihood correct classification, blinding to reference test], and patient flow [relating to test timing, all patients receiving all tests, inclusion of all patients in the analysis]) [6]. We used the AMSTAR 2 tool [7] to critically appraise systematic reviews. Consensus on study quality was reached by discussion by the two reviewers. Data extraction was performed independently by two reviewers (RS, AMS) using standardized forms. In the case of multiple gold standards used within individual studies, we devised the following strategy for abstracting data for calculation of sensitivity and specificity: (a) if multiple molecular confirmatory was performed, but one was listed as the "gold standard," data was abstracted only for the gold standard defined by the original authors; (b) if all specimens were subject to two gold standards, the authors' criteria for defining the gold standard were used (i.e., one or both gold standards positive, as per the original authors); (c) if the authors reported conditional performance of a second gold

standard (i.e., performance of a second-level gold standard test only if a first-level gold standard test was discordant), we preferentially abstracted data relative to the first-level test (since a conditional performance of a second-level gold standard would be unlikely in clinical practice). Furthermore, in the case of studies reporting on multiple types of antibodies used for IHC, we preferentially abstracted data on newer monoclonal antibody preparations over polyclonal antibodies, as the former are currently more favored in clinical practice. The details of the gold standards and IHC antibody type that we used in calculation of diagnostic accuracy were reported. Diagnostic accuracy data was abstracted according to histologic diagnosis to facilitate subgroup analyses.

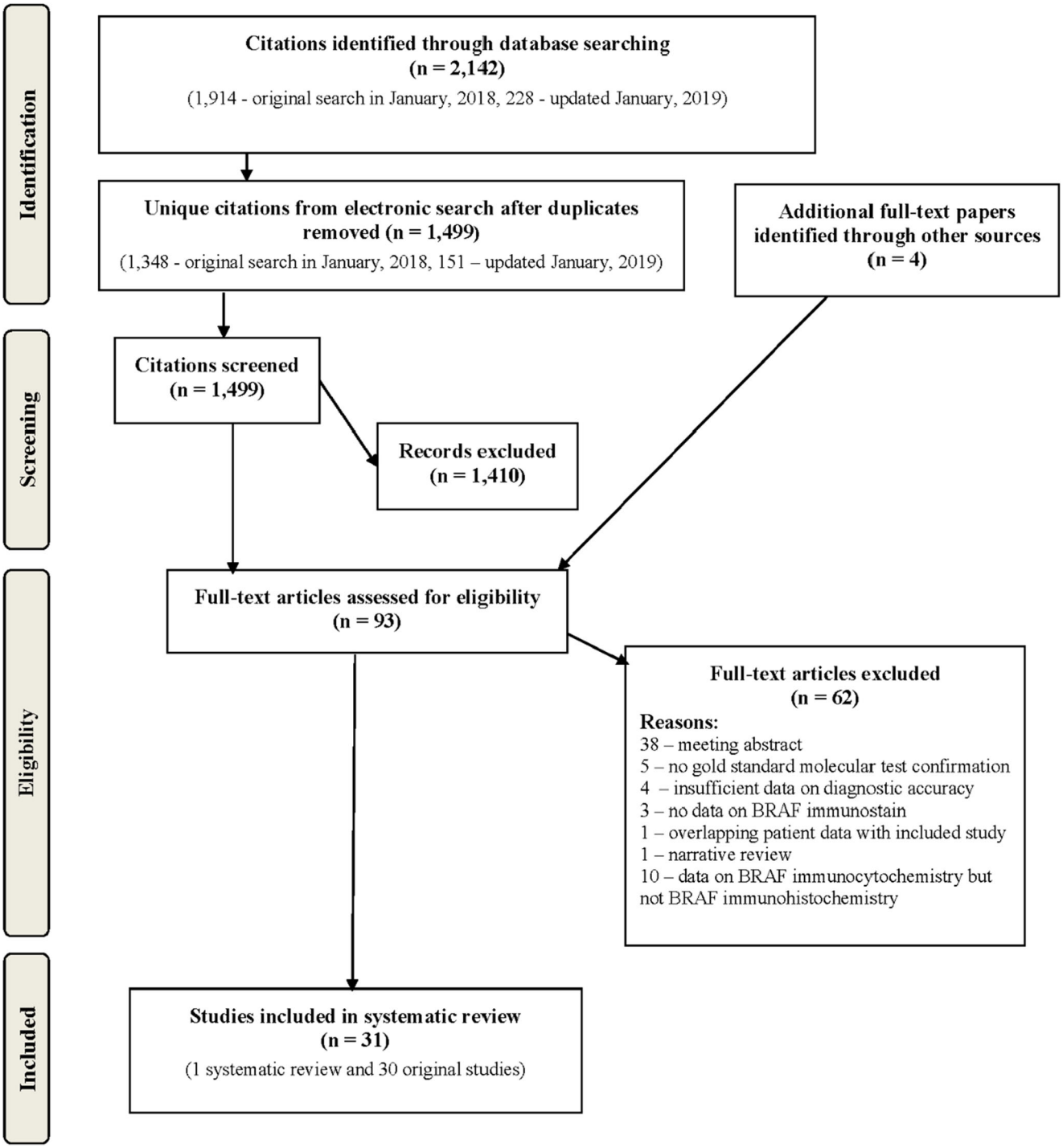

Fig. 1 Study flow diagram

Statistical Analyses

We performed random-effect meta-analyses for the primary analyses of pooled sensitivity and specificity (respectively calculated as pooled prevalence rates, with 95% confidence intervals [CI]) for BRAFV600E IHC in accurately detecting mutation status relative to gold standard molecular test[s]). Random-effect meta-analyses were chosen due to expected study variability according to key variables, including thyroid histologic diagnosis, type of IHC test, and type of gold standard. Histologic subgroup data were incorporated in mixed-effect meta-analyses. Heterogeneity (variability) of diagnostic accuracy results among included studies was evaluated using Cochrane's Q test [8] and the I^2 measure [9]. We evaluated for potential publication bias (bias occurring when favorable results are more likely to be published) the trim and fill method developed by Duval and Tweedie which was applied to diagnostic test meta-analyses by Bürkner and Doebler [10]. The alpha level for statistical significance of heterogeneity was defined as 0.1 using the Cochrane's Q test [9]. Heterogeneity in the primary analyses was analyzed according to the following: (a) type of IHC test, (b) type of gold standard test(s), (c) blinding of the pathologist(s) interpreting the IHC test to the molecular test result, (d) a low risk of bias using QUADAS-2 in all categories, and (e) industry sponsorship or royalties for the IHC test reported to be received by study authors. Sensitivity analyses according to patient or treatment characteristics were not feasible, as data were extracted per histologic specimen, and not per patient. All statistical analyses were performed using the Comprehensive Meta-Analysis software, version 2.0 (Biostat, Inc.).

Results

Results of the Search

We identified 2142 citations through electronic database searching, which yielded 1499 unique citations after removing duplicates (Fig. 1). Additional four relevant full-text papers were identified by the investigators (AMS, OM). We reviewed a total of 93 full-text papers, which ultimately resulted in 31 papers being included [11–41] and 62 papers being excluded (Appendix Table 5; excluded references and reasons for exclusion). The included references included 30 original papers [11–40] and one systematic review published in 2015 by Pyo et al. [41].

Review of the Published Knowledge Synthesis

We retrieved one systematic review and meta-analysis, published by Pyo et al. in 2015 [41], which examined the diagnostic accuracy of BRAFV600E immunohistochemistry using the VE1 clone with *BRAFV600E* mutation testing in papillary thyroid carcinoma (PTC). Pyo et al. included 1141 PTCs in 11 eligible studies, which included immunohistochemistry and immunocytochemistry [41]. Of the 11 studies included by Pyo et al. [41], we also included 7 studies [12, 15,

Table 1 Critical appraisal of the included systematic review and meta-analysis using AMSTAR 2 [7]

Author and year of study: Pyo 2015 [41]	
Quality criteria	Consensus response
1. Study question and inclusion criteria included relevant components?	Yes
2. Explicit statement of pre-established review methods?	No
3. Explanation of the selection of the study design inclusion criteria?	Yes
4. Comprehensive literature search strategy?	Partial Yes
5. Duplicate study selection?	No
6. Duplicate data extraction?	Yes
7. Provision of a list of excluded studies with justification of the exclusions?	No
8. Included studies described in sufficient detail?	Yes
9. Risk of bias of includes studies assessed?	No
10. Sources of funding of included studies reported?	No
11. If meta-analysis was performed, was the statistical method appropriate?	Yes
12. If meta-analysis was performed, was the risk of bias on results evaluated?	No
13. Was the risk of bias of individual studies considered in interpreting and discussing the results?	No
14. Was there a satisfactory explanation and discussion of any heterogeneity of observed results (if present)?	No
15. Was there an evaluation for potential publication bias?	No

Table 2 Characteristics of the included original studies

Study, year [reference]	Country	Setting	Specimen description	BRAF antibody (antibody dilution)	Molecular gold standard test	Study funding and relevant competing interests (e.g., royalties, patent)
Abd Elmageed, 2017 [11]	United States	Louisiana Cancer Research Center	130 PTC surgical specimens	VE1 monoclonal anti-BRAFV600E antibody (1:100 dilution)	Real-time PCR	Academic funding
Bullock, 2012 [12]	Australia	Royal North Shore Hospital	96 PTC surgical specimens	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Sanger sequencing (in discordant cases, repeat IHC and Sanger sequencing plus massive parallel sequencing)	Academic funding, authors received royalties on the VE1 antibody
Capper, 2011 [13]	Germany	University of Heidelberg	18 PTC surgical specimens	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Direct sequencing	Academic funding, authors applied for patent of VE1
Chen, 2018 [14]	China	First Affiliated Hospital of Dalian Medical University	40 PTC surgical specimens, collected in 2011–2013	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Quantitative real-time PCR	No funding reported
Crescenzi, 2014 [15]	Italy	Anatomia Patologica Ospedale Israelitico di Roma	30 Pre-operative core needle biopsies of inconclusive cytology thyroid nodules (20 PTCs, 1 minimally invasive FTC, 9 benign)	VE1 mouse monoclonal anti-BRAFV600E antibody (1:50 dilution)	Pyrosequencing	No funding reported
Da Silva, 2015 [16]	Brazil	Araújo Jorge Hospital	116 PTC surgical specimens (metastatic and non-metastatic), collected in 2000–2005	BRAF F-7 clone monoclonal antibody (1:150 dilution)	Polymerase chain reaction amplification and restriction fragment length polymorphism, confirmed by direct sequencing	Academic funding
De Biase, 2014 [17]	Italy	University of Bologna	20 BRAF-mutated PTCs	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Allele-specific locked nucleic acid PCR and next-generation sequencing	Academic funding
Fisher, 2014 [18]	USA	Emory University	41 Surgical specimens (25 PTC, 1 FTC, 7 MTC, 4 ATC)	VE1 monoclonal anti-BRAFV600E antibody (1:40 dilution)	Pyrosequencing	Not reported
Ghossein, 2013 [19]	USA	Memorial Sloan-Kettering Cancer Center	91 specimens (31 PTC, 38 PDTC, 22 ATC). ATCs from autopsy, rest surgical specimens	VE1 monoclonal anti-BRAFV600E antibody (1:50 dilution)	Mass spectrometry genotyping	Academic funding
Ilie, 2014 [20]	France	Pasteur Hospital	194 PTC surgical specimens consecutively collected in 2004–2012	VE1 monoclonal anti-BRAFV600E antibody (1:10 dilution)	Sanger sequencing, pyrosequencing, and SNaPshot genotyping (one or more positive)	Funding not reported
Jung, 2015 [21]	South Korea	Chung-Ang University Hospital	467 PTC surgical specimens > 4 mm in largest dimension, collected in 2011–2012	VE1 monoclonal anti-BRAFV600E antibody (1:50 dilution)	Real-time PCR and BRAF RNA ISH using tissue microarray (TMA) (unclear if/how tests combined in positive definition)	Academic funding
Kim, 2014 [22]	South Korea	Gang Nam Severance Hospital, Yonsei University	91 PTC surgical specimens collected in 2010–2011	VE1 monoclonal anti-BRAFV600E antibody (1:300 dilution)	Pyrosequencing, peptide nucleic acid-clamping polymerase chain reaction, and real-time polymerase	Academic funding

Table 2 (continued)

Study, year [reference]	Country	Setting	Specimen description	BRAF antibody (antibody dilution)	Molecular gold standard test	Study funding and relevant competing interests (e.g., royalties, patent)
					chain reaction (one or more test positive).	
Kim 2018 [23]	South Korea	Seoul National University Hospital	697 Classic PTC surgical specimens collected in 2013–October 2016, excluding metastatic lymph nodes	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	PCR amplification and direct Sanger sequencing	No funding reported
Lin, 2016 [24]	Taiwan	Taipei Medical University	48 Conventional PTC surgical specimens collected from April 1999 to December 2009	BRAF SC-9002 antibody, (1:200 dilution)	Quantitative real-time PCR	No funding reported
Lin, 2018 [25]	USA	Rush University Medical Centre	7 Specimens of ectopic thyroid tissue collected from November 1992 to June 2016 (6 surgical and 1 autopsy specimen)	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Real-time PCR	No funding reported
Loo, 2017 [26]	USA	Multi-lab: University of New Mexico and TriCore Reference Laboratories	12 Surgical PTC specimens	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Pyrosequencing and next generation sequencing (one or more positive)	No funding reported
Martinuzzi, 2016 [27]	Italy	University of Genoa	86 PTC surgical specimens consecutively collected in 2009–2012	VE1 monoclonal anti-BRAFV600E antibody (1:50 dilution)	PNA-clamp quantitative PCR BRAF detection was reference test (Sanger sequencing also performed).	Academic funding
McKelvie, 2013 [28]	Australia	St Vincent's Hospital	71 PTC surgical cases collected in 1998–2011	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Competitive PCR, For discordant cases: SNaPshot PCR	Ventana Medical Systems provided reagents, loaned equipment and partly funded salary of some investigators to conduct VE1 studies
Na, 2015 [29]	South Korea	Multi-lab: Chonnam National University Hwasun Hospital and Chonnam National University Hospital	141 Thyroid cancer surgical specimens collected in 2005–2013 (104 PTC, 9 ATC, 8 FTC, 20 MTC)	VE1 monoclonal anti-BRAFV600E antibody (1:100 dilution)	Real-time PCR. For discordant cases: repeated IHC and real-time PCR and performed nested PCR and direct sequencing.	Academic funding
Oh, 2018 [30]	South Korea	University of Ulsan College of Medicine	23 Thyroid surgical specimens, 25 preoperative core needle biopsies, separately reported. Patients underwent surgery in 2011–2013. Histologic diagnoses included PTC, FTC, and follicular hyperplasia (subgroup data not reported)	VE1 monoclonal anti-BRAFV600E antibody (1:4 dilution)	Sanger sequencing	Academic funding
Paja Fano, 2017 [31]	Spain	Hospital Universitario de Basurto	82 Consecutive PTC surgical specimens collected between November, 2014, and May, 2016.	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	RT-PCR	No funding reported
Qiu, 2015 [32]	China	Cancer Hospital of the Chinese Academy of Medical		VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)		Academic funding

Table 2 (continued)

Study, year [reference]	Country	Setting	Specimen description	BRAF antibody (antibody dilution)	Molecular gold standard test	Study funding and relevant competing interests (e.g., royalties, patent)
		Sciences, from July 2010 to June 2014	127 Surgical PTC specimens collected between July, 2010, and June, 2014		Sanger sequencing and real-time PCR (both required to be positive)	
Routhier 2013 [33]	USA	Massachusetts General Hospital of Harvard Medical School	23 Surgical thyroid cancer specimens (19 PTC, 4 FTC) collected between, July, 2009, and May, 2012	VE1 monoclonal anti-BRAFV600E antibody (1:100 dilution)	SNaPshot genotyping assay	No funding reported
Rushton, 2016 [34]	United Kingdom	Institution/lab where specimens collected or tests executed not reported	53 ATCs, including 41 ATC surgical specimens and 12 ATC core biopsies, reported together	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Pyrosequencing assay	No funding reported
Sun, 2015 [35]	China	Peking Union Medical College Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College	556 PTC surgical specimens consecutively collected in 2010–2012	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Real-time PCR	No funding reported
Szymonek, 2017 [36]	Poland	Holycross Cancer Center	140 Classical PTC surgical specimens collected in 2000–2005	VE1 monoclonal anti-BRAFV600E antibody (1:100 dilution)	Real-time PCR was reported as gold standard. Sanger sequencing reported as a supplemental test.	Academic funding
Takada, 2018 [37]	Japan	Kuma Hospital	7 PTCs with Desmoid-type fibromatosis surgical specimens collected in 2007–2016	VE1 monoclonal anti-BRAFV600E antibody (1:100 dilution)	PCR and automated capillary DNA sequencing	No funding reported
Zagzag, 2013 [38]	United States	Division of Endocrine Surgery at New York University Langone Medical Center	37 Consecutive classical variant PTC surgical specimens (tumor > 1 cm) from total thyroidectomies and elective central (but not lateral) lymph node dissection	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Direct sequencing	Academic funding
Zhang, 2018 [39]	China	Affiliated Hospital of Qingdao University, Qingdao, China	132 PTC surgical specimens consecutively collected between September, 2013, and February, 2017.	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Amplification refractory mutation system (ARMS)-PCR, for discordant cases, Sanger sequencing.	Academic funding
Zhu, 2016 [40]	China	Fudan University Shanghai Cancer Center	Surgical specimens collected in 2008–2010, including 118 PTCs, 116 benign thyroid tissue, 20 FTCs, 20 MTCs, 20 ATCs	VE1 monoclonal anti-BRAFV600E antibody (dilution not reported)	Sanger sequencing. For discordant cases, amplification refractory mutation system RT-PCR.	Academic funding

PTC, papillary thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer; FTC, follicular thyroid cancer; PCR, polymerase chain reaction

Table 3 Critical appraisal of methodologic quality of included original studies

Study, year [reference]	Risk of bias in patient/ specimen selection	Risk of bias immunohistochemistry test conduct or interpretation	Risk of bias molecular gold standard conduct or interpretation	Risk of bias due to patient or test timing or flow
Abd Elmageed, 2017 [11]	High	Low	Low	Low
Bullock, 2012 [12]	Low	Low	High	High
Capper, 2011 [13]	High	Low	Low	High
Chen, 2018 [14]	High	High	Low	Low
Crescenzi, 2014 [15]	High	Low	Low	Low
DaSilva, 2015 [16]	High	High	Low	Low
De Biase, 2014 [17]	High	High	Low	Low
Fisher, 2014 [18]	High	Low	Low	Low
Ghossein, 2013 [19]	Low	Low	Low	Low
Ilie, 2014 [20]	Low	Low	Low	Low
Jung, 2015 [21]	Low	High	Low	Low
Kim, 2014 [22]	High	High	Low	Low
Kim 2018 [23]	Low	High	Low	Low
Lin, 2016 [24]	Low	High	Low	Low
Lin, 2018 [25]	Low	High	Low	Low
Loo, 2017 [26]	High	High	Low	Low
Martinuzzi, 2016 [27]	Low	Low	Low	Low
McKelvie, 2013 [28]	Low	High	High	High
Na, 2015 [29]	Low	Low	High	High
Oh, 2018 [30]	High	Low	Low	Low
Paja Fano, 2017 [31]	Low	High	Low	Low
Qiu, 2015 [32]	Low	High	Low	Low
Routhier, 2013 [33]	Low	High	Low	Low
Rushton, 2016 [34]	High	High	Low	Low
Sun, 2015 [35]	Low	High	Low	Low
Szymonek, 2017 [36]	Low	Low	Low	Low
Takada, 2018 [37]	Low	High	Low	Low
Zagzag, 2013 [38]	Low	Low	Low	Low
Zhang, 2018 [39]	Low	Low	High	High
Zhu, 2016 [40]	High	High	Low	High

20–22, 28, 38]; however, we excluded 3 papers that exclusively focused on immunocytochemistry (Appendix Table 2; Routhier 2013, Rossi et al. 2014, Zimmerman et al. 2014) as well as one study (Appendix Table 2; Dvorak 2014) in which the study population overlapped with that of another included study of McKelvie et al. [28]. Table 1 summarizes the critical appraisal of this systematic review. Pyo et al. did not critically appraise the quality of the included studies or incorporate this feature in the data analysis or interpretation [41]. The findings of Pyo et al. included a pooled IHC sensitivity of 97% (95% CI, 95%, 98%, with evidence of significant heterogeneity, $I^2 = 50.8\%$) and the specificity of 78% (95% CI, 72%, 83%, also with evidence of significant heterogeneity, $I^2 = 81.4\%$). Given that a larger number of studies were currently available for analysis (30 included in our review [1–30]) and that more published data have become available for non-PTC thyroid malignancies, updating of the review by Pyo et al. [41] was warranted.

Description of the Included Original Studies

The details of the 30 included original studies included in the meta-analyses are reported in Table 2. The regions where studies were conducted included the following: the United States (7 studies) [11, 18, 19, 25, 26, 33, 38], Australia (2 studies) [12, 28], China (5 studies) [14, 32, 35, 39, 40], South Korea (5 studies) [21–23, 29, 30], Europe (8 studies) [13, 15, 17, 20, 27, 31, 34, 36], Brazil [16], Taiwan [24], and Japan [37]. The VE1 monoclonal antibody was used in 28/30 studies [11–15, 17–23, 25–40], whereas Da Silva et al. used a BRAF F-7 monoclonal antibody clone [16] and Lin et al. used a BRAF SC-9002 antibody [24]. The most common thyroid

malignancy was PTC, which was studied in 28/30 studies [11–24, 26–33, 35–40]. Molecular gold standards were highly variable (see Table 2). The majority of studies were either academically funded or unfunded without significant competing interests (such as royalties to one or more authors) [11, 14–27, 29–40]. The critical appraisal of methodologic quality of the 30 studies included in the meta-analyses is described in Table 3. Approximately 40% (12/40) of the studies were judged to be at high risk of bias in patient/specimen selection (e.g., non-consecutive or non-random patient selection or unclear method of patient selection) [11, 13–18, 22, 26, 30, 34, 40]. Furthermore, more than half of the studies (56.7%) were at high risk of bias in IHC test conduct or interpretation due to lack of reported blinding to the gold standard result or lack of a clearly pre-specified definition of IHC positivity threshold (relative to the intensity of staining) [14, 16, 17, 21–26, 28, 31–35, 37, 40]. However, the risk of bias of the molecular gold standard conduct or interpretation was judged as high in a minority of studies, where a discordant result between IHC and a gold standard prompted testing by another gold standard, and, therefore, there would be knowledge of some discordance of the first test when the second test would be performed (13.3%, 4/30) [12, 28, 29, 39]. High risk of bias due to patient or test timing or flow was judged to be present in 23.3% of studies (7/30) [12, 13, 28, 29, 39, 40] due to factors such as not all patients/specimens receiving the reference standard(s), not all patients receiving the same reference standard(s), or relatively high rate of exclusion of patients/specimens from the analysis.

Results of the Meta-Analyses

In a random-effect meta-analysis, the pooled sensitivity of IHC in detecting a *BRAFV600E* mutation was 96.8% (95% confidence interval [CI] 94.1%, 98.3%) (including data from 33 histologic subgroups in 29 studies, 2659 *BRAFV600E*

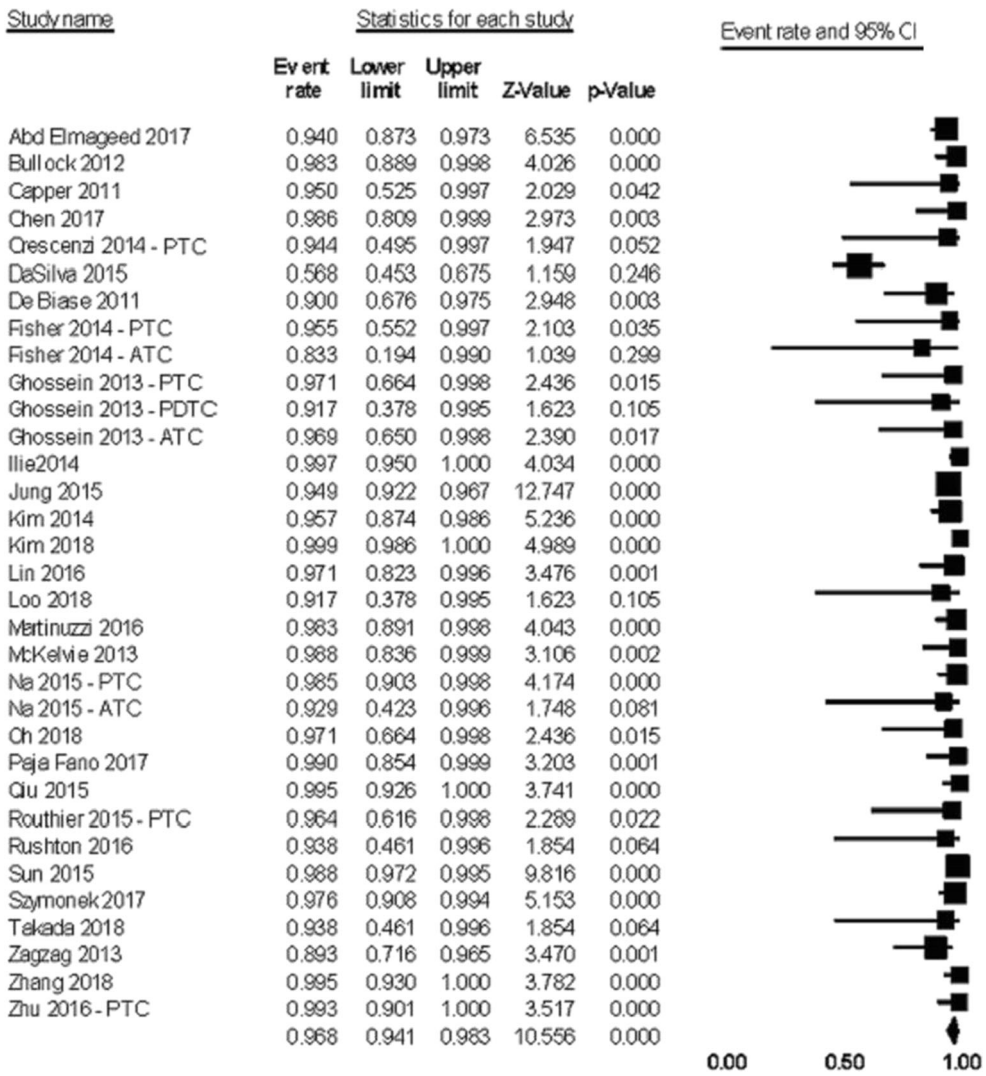

Fig. 2 Forest plot from a random-effect meta-analysis examining sensitivity of BRAF immunohistochemistry in detecting the *BRAFV600E* mutation in thyroid histopathology

mutant tumors, subject to statistically significant heterogeneity, $Q = 159.5$, $p < 0.001$, degrees of freedom [df] = 32, $I^2 = 79.9$) (Fig. 2). The pooled specificity of IHC was 86.3% (95% CI 80.7%, 90.4%) (including data from 39 subgroups in 28 studies, including 1107 *BRAFV600E* wild-type specimens, with statistically significant heterogeneity, $Q = 124.6$, $p < 0.001$, degrees of freedom [df] = 38, $I^2 = 69.5$) (Fig. 3). Analyses adjusted for potential publication bias using a random-effect trim and fill method yielded the following results: sensitivity at 95.2% (95% CI 91.7%, 97.3%, with 7 studies trimmed) and specificity at 77.8% (70.2%, 84.0%, with 13 studies trimmed).

Various categories of subgroups were compared to explore potential explanations for heterogeneity in both sensitivity and specificity meta-analyses. Regarding IHC sensitivity, heterogeneity in results was partly explained by differences in performances of IHC tests (VE1 clone: 96.3%, 95% CI 95.2%, 97.1%; F-7 clone: 56.8%, 95% CI 45.3%, 67.5%; SC-9002: 97.1%, 95% CI 82.3%, 99.6%; between-group difference $Q = 33.5$, df = 2, $p < 0.001$). However, there were no significant between-group differences in sensitivity according to the following: (a) histologic subtype (PTC, anaplastic thyroid cancer [ATC], poorly differentiated thyroid cancer [PDTC], and mixed histology subgroups, $Q = 1.0$, df = 3, $p = 0.814$); (b) type of molecular gold standard (gold standard including sequencing [of any type] of all specimens, gold standard not including any sequencing, or two-tiered testing with a second-level test in the case of discordant first-level testing, $Q = 2.2$, df = 2, $p = 0.340$); (c) presence or absence of industry funding or relevant competing interests (e.g.,

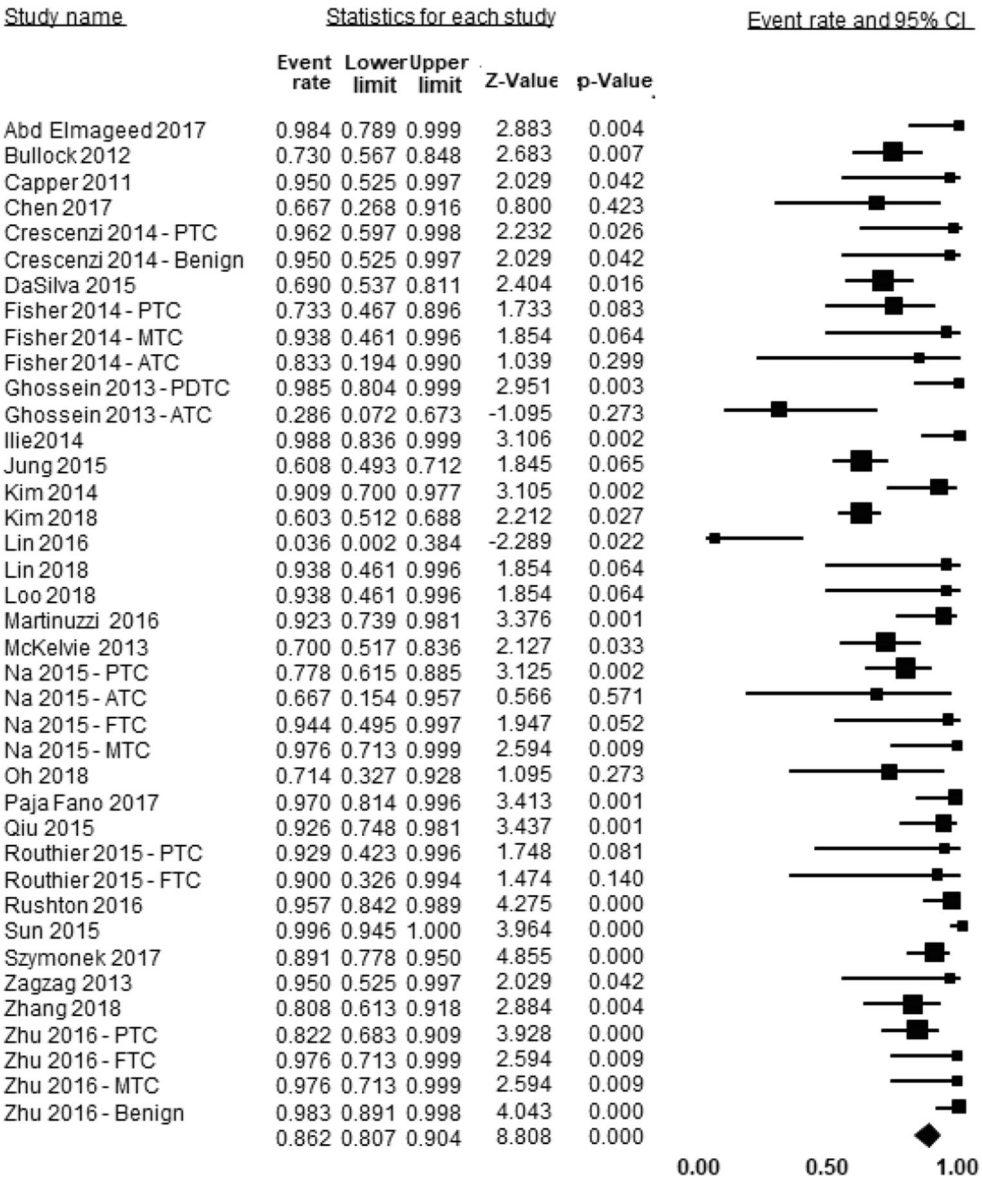

Fig. 3 Forest plot from a random-effect meta-analysis examining specificity of BRAF immunohistochemistry in thyroid histopathology

patents, royalties) ($Q = 0.2$, df = 1, $p = 0.687$); (d) reported blinding of pathologists interpreting IHC to the molecular test result(s) ($Q = 0.02$, df = 1, $p = 0.879$); or (e) low risk of bias in all categories of QUADAS-2 ($Q = 0.6$, df = 1, $p = 0.430$). The statistical heterogeneity in the BRAF V600E IHC meta-analysis was partly explained by significant between-group differences according to histologic diagnosis (PTC, ATC, PDTC, mixed histology subgroup, follicular thyroid cancer [FTC], benign thyroid tissue, ectopic benign thyroid tissue, $Q = 12.2$, df = 7, $p = 0.095$) as well as type of BRAF antibody (VE1 clone: 87.7%, 95% CI 82.4%, 91.6%; F-7 clone: 69.0%, 95% CI 25.1%, 93.7%; SC-9002: 3.6%, 95% CI 0.1%, 51.0%; between-group difference $Q = 10.6$, df = 2, $p = 0.005$). However, there were no significant between-group differences in specificity according to the following: (a) type of molecular gold standard (gold standard including sequencing [of any type] of all specimens, gold standard not including any sequencing, or two-tiered testing with a second-level test in the case of discordant first-level testing, $Q = 0.1$, df = 2, $p = 0.962$); (b) presence or absence of industry funding or relevant competing interests (e.g., patents, royalties) ($Q = 1.0$, df = 1, $p = 0.320$); (c) reported blinding of pathologists interpreting IHC to the molecular test result(s) ($Q = 0.4$, df = 1, $p = 0.513$); or (d) low risk of bias in all categories of QUADAS-2 ($Q = 0.8$, df = 1, $p = 0.360$). The sensitivity and specificity of BRAF V600 E IHC are grouped according to histologic diagnosis in Table 4.

Discussion

In this study, the analytic validity of IHC in detecting the *BRAFV600E* mutation in thyroid histopathology was estimated by a pooled sensitivity at 96.8% (95% CI 94.1%, 98.3%) and pooled specificity at 86.3% (95% CI 80.7%, 90.4%) (data from 30 original studies, including 2659 *BRAFV600E* mutant and 1107 wild-type specimens). These meta-analyses were subject to statistically significant heterogeneity, partly explained by antibody type (for both the sensitivity and specificity meta-analyses) and tissue/tumor type (for the specificity meta-analysis). Our analytic validity meta-analyses generally confirm those previously reported in PTC specimens by Pyo et al. [41]. However, our review included data on other types of thyroid malignancies and control specimens. Our study supports that of prior research, indicating that the choice of antibody significantly impacts BRAF IHC analytic performance. For example, Fisher et al. reported that the specificity of the VE1 antibody was superior to that of pan-BRAF [18]. We did not compare specific details of IHC protocols (other than antibody used) nor the quality of preservation of histopathologic specimens, which may account for some of the unexplained variability in results.

The strengths of this work include a systematic search for relevant citations in multiple databases by an experienced library information specialist, independent review of citations and full-text papers by two reviewers, and duplicate,

Table 4 Mixed (random)-effect meta-analyses of sensitivity and specificity of BRAF V600E immunohistochemistry according to histologic diagnosis

Histologic diagnosis	Estimate	Number of studies (number of specimens) [references]	Point estimate, 95% confidence interval			Heterogeneity			
			Point estimate (%)	Lower limit (%)	Upper limit (%)	Q value	df (Q)	p value	I^2
PTC	Sensitivity	27 Studies (2608 BRAF mutant) [11–24, 26–29, 31–33, 35–40]	97.2	94.4	98.6	158.2	26	< 0.001	83.6
	Specificity	24 Studies (854 wild-type) [11–16, 18, 20–24, 26–29, 31–33, 35, 36, 38–40]	83.1	75.9	88.5	80.8	23	< 0.001	71.6
ATC	Sensitivity	4 Studies (30 BRAF mutant) [18, 19, 29, 34]	93.1	63.1	99.1	0.8	3	0.86	0
	Specificity	4 Studies (58 BRAF mutant) [18, 19, 29, 34]	76.4	47.5	92.1	13.4	3	0.004	77.7
PDTC	Sensitivity	1 Study (5 BRAF mutant) [19]	91.7	15.2	99.9	N/A	N/A	N/A	N/A
	Specificity	1 Study (33 wild-type) [19]	98.5	72.9	99.9	N/A	N/A	N/A	N/A
Mixed types	Sensitivity	1 Study (16 BRAF mutant) [30]	97.1	36.3	99.9	N/A	N/A	N/A	N/A
	Specificity	1 Study (7 wild-type) [30]	71.4	20.2	96.1	N/A	N/A	N/A	N/A
FTC	Specificity	3 Studies (32 wild-type) [29, 33, 40]	94.9	73.9	99.2	0.5	2	0.761	0
MTC	Specificity	3 Studies (47 wild-type) [18, 29, 40]	96.7	81.9	99.5	0.3	2	0.852	0
Benign thyroid	Specificity	2 Studies (69 wild-type) [15, 40]	97.5	83.8	99.7	0.4	1	0.521	0
Ectopic thyroid (benign)	Specificity	1 Study (7 wild-type) [29]	93.8	36.1	99.7	N/A	N/A	N/A	N/A

N/A, within-group heterogeneity calculation not applicable as there was only one study in the group; PTC, papillary thyroid cancer; PDTC, poorly differentiated thyroid cancer; ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer; FTC, follicular thyroid cancer

independent data abstraction, and quality appraisal of included studies. Some limitations of this review include limited searching of the gray literature and exclusion of non-English studies. Furthermore, there was some evidence of potential publication bias (particularly for specificity) as well as heterogeneity in the meta-analyses, the latter of which was not fully explained.

The results of this review confirm that *BRAFV600E* IHC, using the VE1 antibody in PTC specimens, is highly sensitive, such that a negative test largely excludes this mutation. This finding may be helpful in the classification of lesions that appear to have a predominant follicular pattern but may have subtle features that raise the possibility of classical variant PTC, such as small abortive papillae. The application of *BRAFV600E* IHC in multifocal thyroid cancer may also enable detailed characterization of respective lesions. However, given the critical need for accurate documentation of a *BRAF* mutation when considering targeted therapy as well as some observed limitations in IHC specificity (e.g., 76% in ATC specimens observed in this review), confirmatory molecular testing may be considered in *BRAFV600E* IHC-positive cases, if it is feasible and critical treatment would not be excessively delayed. Further research is needed to ensure implementation of optimal laboratory validation of *BRAFV600E* IHC testing protocols and reduce variability in results. Clinical utility research examining the long-term patient outcome impact of routine use of *BRAFV600E* IHC is also needed.

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Compliance with Ethical Standards

Conflict of Interest None declared.

Appendix 1 Sample Search Strategy

Database: Ovid MEDLINE: Epub ahead of print, in-process, and other non-indexed citations, Ovid MEDLINE® Daily and Ovid MEDLINE® <1946–Present>

1 1 exp thyroid neoplasms/
2 (thyroid adj cancer$).mp.
3 (thyroid adj carcinoma$).mp.
4 (thyroid adj neoplasm$).mp.
5 (thyroid adj adenoma$).mp.
6 (thyroid adj tumo?r$).mp.
7 or/1–6
8 BRAF$.mp.
9 B-raf$.mp.
10 “proto-oncogene B-Raf”.mp.
11 NS7.mp.
12 “p94”.mp.
13 “proto-oncogene B-Raf”.mp.
14 “murine sarcoma viral (v-raf) oncogene homolog B1”.mp.
15 RAFB1.mp.
16 “94 kDa B-raf protein”.mp.
17 “v-raf murine sarcoma viral oncogene homolog B”.mp.
18 “v-raf murine sarcoma viral oncogene homolog B1”.mp.
19 or/8–18
20 exp. Immunohistochemistry/
21 Histocytochemistry/
22 Biomarkers, Tumor/
23 “Staining and Labeling”/
24 immunohistochem$.mp.
25 histocytochem$.mp.
26 immunocytochem$.mp.
27 immunohistocytochem$.mp.
28 immunostain$.mp.
29 biomarker$.mp.
30 histochem$.mp.
31 marker$.mp.
32 stain$.mp.
33 (mark$ adj3 agent$).mp.
34 (antibod$ adj3 label$).mp.
35 (immun$ adj3 label$).mp.
36 immunolabel$.mp.
37 or/20–36
38 7 and 19 and 37
39 exp. animals/not (exp animals/and humans/)
40 38 not 39
41 limit 40 to yr=“2005–Current”

Appendix 2

Table 5 Details of the full-text papers that were reviewed and excluded (with reasons for exclusion)

No gold standard molecular test confirmation of *BRAFV600E* mutation	Zhao L, Jiang R, Xu M, Zhu P, Mo XM, Wang N, Chen GG, Liu ZM (2016) Concomitant high expression of BRAFV600E, P-cadherin and cadherin 6 is associated with High TNM stage and lymph node metastasis in conventional papillary thyroid carcinoma. Clinical Endocrinology 84:748–55.
	Zhang X, Chen S, De J, Wenjuan W (2017) Expressions of Slug, BRAF V600E and STIP1 proteins and their correlation with capsular invasion and regional lymph node metastasis in papillary thyroid carcinoma. Cancer Research and Clinic 29:104–7.
	Feng L, Li M, Zhang QP, Piao ZA, Wang ZH, Lv S (2011) Utility of BRAF protein overexpression in predicting the metastasis potential of papillary thyroid carcinoma. Oncology Letters. 2(1):59–63.
	Barabadze E, Munjishvili V, Burkadze G (2017) Braf Antibody Expression in Different Types of Thyroid Nodular Lesions. Georgian Medical News 271:107–113.
	Bai Y, Niu D, Huang X, Jia L, Kang Q, Dou F, Ji X, Xue W, Liu Y, Li Z, Feng Q, Lin D, Kakudo K (2017) PD-L1 and PD-1 expression are correlated with distinctive clinicopathological features in papillary thyroid carcinoma. Diagnostic Pathology 12(1):72.
Meeting abstract	Yi JW, Kim JK, Seong CY, Bae IE, Yu HW, Kim SJ, Chai YJ, Choi JY, Lee KE (2017) Comparison of immunohistochemistry and direct sequencing methods for identify BRAFV600E mutation in papillary thyroid carcinoma. Langenbeck's Archives of Surgery 402(2): 378.
	Yang P, Lum H, Quek J, Kyu H, Lim C, Koay E, Parameswaran R, Nga M (2015) Braf immunohistochemistry score predicts BRAF V600E mutational status in classical papillary thyroid cancer. Thyroid 1):A374–5.
	Wobker S, Kim L, Hackman T, Dodd L (2015) Use of braf immunohistochemistry on cytologic direct smears of papillary thyroid carcinoma. Laboratory Investigation 1): 112A.
	Weber E, Ringelband R, Strumpf A, Kotzerke J, Baretton GB, Freitag M, Zietz C (2012) BRAF V600E is associated with overexpression of cyclin D1, CK 19 and with distinct histomorphological features in papillary thyroid carcinoma. Langenbeck's Archives of Surgery 397(7):1189–90.
	Temprana-Salvador J, Hernandez-Losa J, Zafon C, Camacho J, Diaz S, Ramos I, Somoza R, Strohecker I, Gonzalez O, Garcia-Burillo A, Cajal SRY, Iglesias C (2016) Correlation between immunohistochemical BRAF V600E expression and mutational status detected by two different PCR based molecular techniques in papillary thyroid carcinomas. Laboratory Investigation 1):522A.
	Solomides CC, Draganova-Tacheva R, Bibbo M, Ren S, Tuluc M, Birbe R, Wang ZX, Peiper SC (2014) The utility of BRAF V600E monoclonal mutation specific antibody for detection of BRAF V600E mutation on cell block specimens of thyroid fine needle aspiration. Laboratory Investigation 1):122A.
	Smith AL, Williams MD, Cabanillas ME, Stewart J, Wang WL, Krishnamurthy S, Roy-Chowdhuri S (2017) Utility of BRAF V600E immunoperoxidase stain in cytology preparations of cell block and aspirate smears in thyroid cancers. Laboratory Investigation 97 (Supplement 1):117A-8A.
	Rossle M, Zimmermann AK, Zimmermann D, Bode B (2013) Value of immunohistochemical detection of BRAFV600E-mutated protein in fine needle aspiration biopsies of papillary thyroid carcinoma. Acta Cytologica 1):32.
	Rossi E, Martini M, Straccia P, Capodimonti S, Lanza P, Lombardi C, Pontecorvi A, Larocca LM, Fadda G (2013) The sequential application of immunocytochemistry, braf-1 and n-ras mutation analysis identifies malignant follicular thyroid neoplasms on liquid-based cytology. Acta Cytologica 1): 135.
	Rossi ED, Martini M, Straccia P, Capodimonti S, Lombardi CP, Pontecorvi A, Vellone VG, Zannoni GF, Larocca LM, Fadda G (2013) The sequential application of immunocytochemistry, BRAF-1 and N-RAS mutation analysis identifies malignant follicular thyroid neoplasms on liquid-based cytology. Laboratory Investigation 1):102A.
	Rossi ED, Martini M, Straccia P, Capodimonti S, Lombardi CP, Pontecorvi A, Larocca LM, Fadda G (2012) Immunocytochemistry and braf-1 mutation analysis identify high-risk follicular neoplasms on liquid based cytology. Cytopathology 1):73.
	Paulson V, Howitt B, Barletta J (2015) Absence of BRAF V600E supports the indolent nature of non-infiltrative, non-invasive follicular variant of papillary thyroid carcinoma. Laboratory Investigation 1):140A.
	Nonaka D, Rushton S, Burghel G, Wallace A (2015) BRAF V600E mutation status in anaplastic thyroid carcinoma. Virchows Archiv 1):S71.
	Nonaka D, Rushton S, Burghel G, Wallace A (2015) BRAF V600E mutation status in anaplastic thyroid carcinoma. Laboratory Investigation 1):139A.
	Murro D, Javidiparsijani S, Vardouniotis A, Buckingham L, Gattuso P (2016) BRAF and RAS mutation analysis to distinguish benign ectopic thyroid tissue and metastatic thyroid carcinoma. Laboratory Investigation 1):152A.

Table 5 (continued)

John IY, Valente A, Tull J, Maciak C, Zhang S (2015) Potential pitfalls in the detection of BRAF v600e mutation by immunohistochemistry. Laboratory Investigation 1):515A-6A.

John I, Tull J, Maciak C, Zhang S (2014) Detection of BRAF V600E mutant protein expression by immunohistochemistry in malignant and benign thyroid lesions. Laboratory Investigation 1):154A.

Rossi ED, Martini M, Angrisani B, Straccia P, Bizzarro T, Ricci C, Pontecorvi A, Lombardi CP, Larocca LM, Fadda G (2014) The comparative analysis of molecular BRAF V600E with the intensity of immunocytochemical positivity in prospective thyroid malignant FNACS. Laboratory Investigation 1):119A.

Rossi E, Martini M, Capodimonti S, Straccia P, Bizzarro T, Ricci C, Lombardi C, Pontecorvi A, Larocca L, Fadda G (2014) Role of immunocytochemical and molecular BRAF expression in thyroid neoplasms: A cyto-histological institutional experience. Virchows Archiv 1):S49.

Rossi E (2013) Pre-analytic steps for BRAF testing on thyroid fine needle aspirations. Acta Cytologica 1):11.

Hung MR, Liu SF, Hang JF, Hsu CY, Lai CR (2016) BRAF VE1 immunocytochemistry on cytospin prepared slides using residual liquid-based cytology materials from papillary thyroid carcinoma fine needle aspirations. Acta Cytologica. 60 (Supplement 1): 225.

Han EJ, Sica GL, Zhang C, Chen Z, Lawson D, Saba NF, Shin DM, Khuri FR, Cohen C, Owonikoko TK (2015) Thyroid cancer outcome and association with immune checkpoint proteins, programmed cell death 1 (PD-1) and PD-1 ligand (PD-L1), lymphocytic infiltrates subsets and mutant B-Raf protein expression. Thyroid 1):A137.

Ghossein R, Ricarte-Filho J, Knauf J, Rivera M, Fagin J (2013) Immunohistochemical (IHC) detection of mutated BRAFV600E along the spectrum of thyroid carcinoma (TC) progression. Laboratory Investigation 1):132A-3A.

Gamboa-Dominguez A, Tenorio-Serralta M, De Oca DMM (2015) BRAFV600E mutation influence in microscopic papillary thyroid carcinoma-a 10-year follow-up. Laboratory Investigation 1):134A.

Fisher KE, Ehsani L, Neill SG, Caltharp SA, Siddiqui MT, Cohen C (2013) BRAF V600E mutations in thyroid carcinomas: Is there a role for BRAF immunohistochemistry? Laboratory Investigation 1)132A.

Eymerit-Morin C, Buffet C, Cancel-Tassin G, Roger M, Gaffory C, Lesot A, Le Naour G, Herve G, Wassermann J, Khayat D, Tresallet C, Leenhardt L, Menegaux F, Rousselet MC, Capron F, Tissier F (2015) BRAFV600E status in solitary and multiple papillary thyroid carcinoma and in their lymph-node and distant metastases: An immunochemical and genotypic retrospective study of 363 consecutive patients. Laboratory Investigation 1):134A.

Etxezarraga MC, Zufiaurre M, Ugalde A, Nieto JA, Solano JD (2015) Correlation between immunohistochemistry using anti-BRAF V600E (VE1) antibody, and BRAF V600Emutation by RT-PCR with COBAS 4800 in Papillary Thyroid Carcinoma (PTC): A study of twenty-six cases. Virchows Archiv 1):S70.

Erkilic S, Elboga U (2016) BRAF mutation in poorly differentiated thyroid carcinoma. Virchows Archiv 469 (Supplement 1):S77.

Erkilic S, Elboga U (2016) Comparison of immunohistochemistry and real-time PCR in detection of BRAF mutation in papillary thyroid carcinoma. Virchows Archiv 469 (Supplement 1):S77.

Dean C, Geller R, Cohen C, Lewis M, Hanley K (2015) BRAF V600E antibody as an adjunct tool in triaging thyroid fine needle aspiration specimens. American Journal of Clinical Pathology 2):A090.

De Koster EJ, De Geus Oei LF, Dekkers OM, Van Engenvan Grunsven I, Hamming JF, Van Der Kleij-Corssmit EPM, Morreau H, Schepers A, Smit JWA, Oyen WJG, Vriens D (2017) Diagnostic utility of molecular and imaging biomarkers in cytologically indeterminate thyroid nodules: A systematic review and meta-analysis. European Journal of Nuclear Medicine and Molecular Imaging 44(2 Supplement 1):S394.

Lassalle S, Ilie M, Hofman V, Bonnetaud C, Bordonne O, Lamy A, Sabourin JC, Haudebourg J, Butori C, Peyrottes I, Sadoul JL, Bozec A, Santini J, Hofman P (2013) Usefulness of immunohistochemistry for the detection of BRAFV600E mutation in papillary thyroid carcinoma. Comparison with three molecular biology methods (dideoxy sequencing, pyrosequencing and snapshot) Laboratory Investigation 1):134A.

Colato C, Marchetti I, Piccoli P, Montagna L, Di Coscio G, Brazzarola P, Chilosi M, Ferdeghini M (2013) Immunohistochemical assay of the BRAFV600E-mutated protein in papillary thyroid carcinoma and comparison with sequencing analysis. European Thyroid Journal 1):95.

Loo E, Khalili P, Buehler K, Siddiqi I, Vasef M (2015) BRAF v600e mutation in melanoma, colorectal carcinoma, hairy cell leukemia, papillary thyroid carcinoma, and langerhans cell histiocytosis: Concordance between molecular testing and mutation-specific immunohistochemistry. Laboratory Investigation 1):518A-9A.

Dvorak K, Palting JD, Aggeler B (2013) Evaluation of BRAF(V600E) mutation by immunohistochemical staining with anti-BRAF V600E (VE1) antibody: A comparison with Sanger sequencing. Cancer Research. Conference: 104th Annual Meeting of the American Association for Cancer Research, AACR. 73(8 Supplement):1.

Dvorak K, Palting J, Waring PM (2013) Detection of the BRAF V600E mutation in colon cancer and thyroid cancer by immunohistochemistry. Molecular Cancer Therapeutics. Conference: AACR NCI EORTC International Conference: Molecular Targets and Cancer Therapeutics 12(11 Supplement):1.

Diaz-Perez JA, Reddy A, Amaro D (2013) Meta-analysis of the molecular-expression signatures accuracy in cytological indeterminate thyroid nodules. Laboratory Investigation 1):132A.

Table 5 (continued)

	Adackapara CA, Howitt BE, Sholl LM, Krane JF, Hornick JL, Barletta JA (2013) BRAF V600E mutation-specific monoclonal antibody is predictive of BRAF mutation status determined by genotyping in papillary thyroid carcinoma. Laboratory Investigation 1):129A.
No data on BRAF immunostain	Wu H, Sun Y, Ye H, Yang S, Lee SL, de las Morenas A (2015) Anaplastic thyroid cancer: outcome and the mutation/expression profiles of potential targets. Pathology Oncology Research 21:695–701.
	Fluge O, Bruland O, Akslen LA, Lillehaug JR, Varhaug JE (2006) Gene expression in poorly differentiated papillary thyroid carcinomas. Thyroid 16(2):161–75.
	Min HS, Lee C, Jung KC (2013) Correlation of immunohistochemical markers and BRAF mutation status with histological variants of papillary thyroid carcinoma in the Korean population. Journal of Korean Medical Science 28(4):534–41.
Insufficient data on sensitivity, specificity or variables used to calculate these estimates for *BRAFV600E* immunohistochemistry	Meng Z, Lu J, Wu H, Zhao Y, Luo Y, Gao J, Zhu Q, Jiang Y, Li W, Liang Z (2016) Mutant-specific BRAF and CD117 immunocytochemistry potentially facilitate risk stratification for papillary thyroid carcinoma in fine-needle aspiration biopsy specimens. Tumour Biology 37(1):611–8.
	Fraser S, Go C, Aniss A, Sidhu S, Delbridge L, Learoyd D, Clifton-Bligh R, Tacon L, Tsang V, Robinson B, Gill AJ, Sywak M (2016) BRAF(V600E) Mutation is Associated with Decreased Disease-Free Survival in Papillary Thyroid Cancer. World Journal of Surgery 40(7):1618–24.
	Koperek O, Kornauth C, Capper D, Berghoff AS, Asari R, Niederle B, Von Deimling A, Birner P, Preusser M (2012) Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. American Journal of Surgical Pathology 36(6):844–50.
	Kondo T, Nakazawa T, Murata S, Kurebayashi J, Ezzat S, Asa SL, Katoh R (2007) Enhanced B-Raf protein expression is independent of V600E mutant status in thyroid carcinomas. Human Pathology 38(12):1810–8.
Overlapping study population data with another included study	Dvorak K, Aggeler B, Palting J, McKelvie P, Ruszkiewicz A, Waring P (2014) Immunohistochemistry with the anti-BRAF V600E (VE1) antibody: impact of pre-analytical conditions and concordance with DNA sequencing in colorectal and papillary thyroid carcinoma. Pathology 46(6):509–17.
Data on immunocytochemistry, not immunohistochemistry	Zimmermann AK, Camenisch U, Rechsteiner MP, Bode-Lesniewska B, Rossle M (2014) Value of immunohistochemistry in the detection of BRAF(V600E) mutations in fine-needle aspiration biopsies of papillary thyroid carcinoma. Cancer Cytopathology 122(1):48–58.
	Rossi ED, Martini M, Capodimonti S, Straccia P, Cenci, T, Lombardi CP, Pontecorvi A, Larocca LM, Fadda G (2013) Diagnostic and prognostic value of immunocytochemistry and BRAF mutation analysis on liquid-based biopsies of thyroid neoplasms suspicious for carcinoma. European Journal of Endocrinology 168(6):853–9.
	Rossi ED, Martini M, Capodimonti S, Cenci T, Straccia P, Angrisani B, Ricci C, Lanza P, Lombardi CP, Pontecorvi A, Larocca LM, Fadda G (2014) Analysis of immunocytochemical and molecular BRAF expression in thyroid carcinomas: a cytohistologic institutional experience. Cancer Cytopathology 122(7)527–35.
	Rossi ED, Bizzarro T, Martini M, Capodimonti S, Fadda G, Larocca LM, Schmitt F (2014) Morphological parameters able to predict BRAFV600E-mutated malignancies on thyroid fine-needle aspiration cytology: Our institutional experience. Cancer Cytopathology 122(12):883–91.
	Leslie C, Grieu-Iacopetta F, Richter A, Platten M, Murray J, Frost FA, Amanuel B, Kumarasinghe MP (2015) BRAF p.Val600Glu (V600E) mutation detection in thyroid fine needle aspiration cell block samples: a feasibility study. Pathology 47(5):432–8.
	Lee SR, Yim H, Han JH, Lee KB, Lee J, Soh EY, Kim DJ, Chung YS, Jeong S, Sheen SS, Park SH, Kim JH (2015) VE1 antibody is not highly specific for the BRAF V600E mutation in thyroid cytology categories with the exception of malignant cases. Pathology 47(5):432–8.
	Kim YH, Yim H, Lee YH, Han JH, Lee KB, Lee J, Soh EY, Jeong SY, Kim JH (2016) Evaluation of the VE1 Antibody in Thyroid Cytology Using Ex Vivo Papillary Thyroid Carcinoma Specimens. Journal of Pathology & Translational Medicine 50(1):58–66.
	Smith AL, Williams MD, Stewart J, Wang WL, Krishnamurthy S, Cabanillas ME, Roy-Chowdhuri S (2018) Utility of the BRAF p.V600E immunoperoxidase stain in FNA direct smears and cell block preparations from patients with thyroid carcinoma. Cancer Cytopathology 126(6):406–13.
	Choi YD, Park CS, Nam JH, Kim GE, Kim SS (2018) Use of VE1 immunostaining on FNA of papillary thyroid carcinoma. Cytopathology 29(Supplement 1): 72.
Narrative review	Baloch Z, Mete O, Asa SL (2018) Immunohistochemical biomarkers in thyroid pathology. Endocrine Pathology 29(2):91–112.

References

1. Lim H, Devesa SS, Sosa JA, Check D, Kitahara CM. (2017) Trends in thyroid cancer incidence and mortality in the United States, 1974–2013. JAMA. 317(13):1338–1348. https://doi.org/10.1001/jama.2017.2719.
2. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014; 159(3): 676–690.
3. Vuong HG, Altibi AM, Abdelhamid AH, Ngoc PU, Quan VD, Tantawi MY, Elfil M, Vu TL, Elgebaly A, Oishi N, Nakazawa T, Hirayama K, Katoh R, Huy NT, Kondo T. (2017) The changing characteristics and molecular profiles of papillary thyroid carcinoma over time: a systematic review. Oncotarget 8(6):10637–10649. https://doi.org/10.18632/oncotarget.12885.
4. Lloyd RV, Osamura RV, Kloppel G, Rosai J, editors. WHO classification of tumors of endocrine organs (4th edition). IARC: Lyon 2017.
5. Lo MC, Paterson A, Maraka J, Clark R, Goodwill J, Nobes J, Garioch J, Moncrieff M, Rytina E, Igali L. (2016). A UK feasibility and validation study of the VE1 monoclonal antibody immunohistochemistry stain for BRAF-V600E mutations in metastatic melanoma. Br J Cancer 115(2), 223–227. https://doi.org/10.1038/bjc.2016.106.
6. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM; QUADAS-2 Group. (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155(8):529–536. https://doi.org/10.7326/0003-4819-155-8-201110180-00009.
7. Shea BJ, Reeves BC, Wells G, Thuku M, Hamel C, Moran J, Moher D, Tugwell P, Welch V, Kristjansson E, Henry DA. (2017) AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ 358:j4008.https://doi.org/10.1136/bmj.j4008
8. Cochran WG. (1954) The combination of estimates from different experiments. Biometrics 101: 101–129.
9. Higgins JP, Thompson SG, Deeks JJ, Altman DG. (2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–560.https://doi.org/10.1136/bmj.327.7414.557
10. Bürkner PC, Doebler P. (2014) Testing for publication bias in diagnostic meta-analysis: a simulation study. Stat Med 33(18):3061–3067.https://doi.org/10.1002/sim.6177
11. Abd Elmageed ZY, Sholl AB, Tsumagari K, Al-Qurayshi Z, Basolo F, Moroz K, Boulares AH, Friedlander P, Miccoli P, Kandil E. (2017) Immunohistochemistry as an accurate tool for evaluating BRAF-V600E mutation in 130 samples of papillary thyroid cancer. Surgery 161(4):1122–1128.https://doi.org/10.1016/j.surg.2016.06.081.
12. Bullock M, O'Neill C, Chou A, Clarkson A, Dodds T, Toon C, Sywak M, Sidhu SB, Delbridge LW, Robinson BG, Learoyd DL, Capper D, von Deimling A, Clifton-Bligh RJ, Gill AJ. (2012) Utilization of a MAB for BRAF(V600E) detection in papillary thyroid carcinoma. Endocr Relat Cancer 19(6):779–784.https://doi.org/10.1530/ERC-12-0239.
13. Capper D, Preusser M, Habel A, Sahm F, Ackermann U, Schindler G, Pusch S, Mechtersheimer G, Zentgraf H, von Deimling A. (2011) Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. Acta Neuropathol 122(1):11–19. https://doi.org/10.1007/s00401-011-0841-z.
14. Chen D, Qi W, Zhang P, Zhang Y, Liu Y, Guan H, Wang L. (2018) Investigation of BRAFV600E detection approaches in papillary thyroid carcinoma. Pathol Res Pract 214(2):303–307. https://doi.org/10.1016/j.prp.2017.09.001.
15. Crescenzi A, Guidobaldi L, Nasrollah N, Taccogna S, Cicciarella Modica DD, Turrini L, Nigri G, Romanelli F, Valabrega S, Giovanella L, Onetti Muda A, Trimboli P. (2014) Immunohistochemistry for BRAF(V600E) antibody VE1 performed in core needle biopsy samples identifies mutated papillary thyroid cancers. Horm Metab Res 46(5):370–374. https://doi.org/10.1055/s-0034-1368700.
16. da Silva RC, de Paula HS, Leal CB, Cunha BC, de Paula EC, Alencar RC, Meneghini AJ, Silva AM, Gontijo AP, Wastowski IJ, Saddi VA. (2015) BRAF overexpression is associated with BRAF V600E mutation in papillary thyroid carcinomas. Genet Mol Res 14(2):5065–5075. https://doi.org/10.4238/2015.
17. de Biase D, Cesari V, Visani M, Casadei GP, Cremonini N, Gandolfi G, Sancisi V, Ragazzi M, Pession A, Ciarrocchi A, Tallini G. (2014) High-sensitivity BRAF mutation analysis: BRAF V600E is acquired early during tumor development but is heterogeneously distributed in a subset of papillary thyroid carcinomas. J Clin Endocrinol Metab 99(8):E1530–8. https://doi.org/10.1210/jc.2013-4389.
18. Fisher KE, Neill SG, Ehsani L, Caltharp SA, Siddiqui MT, Cohen C. (2014) Immunohistochemical Investigation of BRAF p.V600E mutations in thyroid carcinoma using 2 separate BRAF antibodies. Appl Immunohistochem Mol Morphol 22(8):562–567. https://doi.org/10.1097/PAI.0b013e3182a2f75f.
19. Ghossein RA, Katabi N, Fagin JA. (2013) Immunohistochemical detection of mutated BRAF V600E supports the clonal origin of BRAF-induced thyroid cancers along the spectrum of disease progression. J Clin Endocrinol Metab 98(8):E1414–E1421. https://doi.org/10.1210/jc.2013-1408.
20. Ilie MI, Lassalle S, Long-Mira E, Bonnetaud C, Bordone O, Lespinet V, Lamy A, Sabourin JC, Haudebourg J, Butori C, Guevara N, Peyrottes I, Sadoul JL, Bozec A, Santini J, Capper D, von Deimling A, Emile JF, Hofman V, Hofman P. (2014) Diagnostic value of immunohistochemistry for the detection of the BRAF(V600E) mutation in papillary thyroid carcinoma: comparative analysis with three DNA-based assays. Thyroid 24(5):858–866. https://doi.org/10.1089/thy.2013.0302.
21. Jung YY, Yoo JH, Park ES, Kim MK, Lee TJ, Cho BY, Chung YJ, Kang KH, Ahn HY, Kim HS. (2015) Clinicopathologic correlations of the BRAFV600E mutation, BRAF V600E immunohistochemistry, and BRAF RNA in situ hybridization in papillary thyroid carcinoma. Pathol Res Pract 211(2):162–170.https://doi.org/10.1016/j.prp.2014.10.005.
22. Kim YH, Choi SE, Yoon SO, Hong SW. (2014) A testing algorithm for detection of the B-type Raf kinase V600E mutation in papillary thyroid carcinoma. Hum Pathol 45(7):1483–1488. https://doi.org/10.1016/j.humpath.2014.02.025.
23. Kim JK, Seong CY, Bae IE, Yi JW, Yu HW, Kim SJ, Won JK, Chai YJ, Choi JY, Lee KE. (2018) Comparison of immunohistochemistry and direct sequencing methods for identification of the BRAF(V600E) mutation in papillary thyroid carcinoma. Ann Surg Oncol 25(6):1775–1781. https://doi.org/10.1245/s10434-018-6460-3.
24. Lin JD, Fu SS, Chen JY, Lee CH, Chau WK, Cheng CW, Wang YH, Lin YF, Fang WF, Tang KT. (2016) Clinical manifestations and gene expression in patients with conventional papillary thyroid carcinoma carrying the BRAF(V600E) mutation and BRAF pseudogene. Thyroid 26(5):691–704. https://doi.org/10.1089/thy.2015.0044.
25. Lin DM, Javidiparsijani S, Vardouniotis A, Buckingham L, Reddy SB, Gattuso P. (2018) Ectopic thyroid tissue: Immunohistochemistry and molecular analysis. Appl Immunohistochem Mol Morphol 26(10):734–739. https://doi.org/10.1097/PAI.0000000000000515.
26. Loo E, Khalili P, Beuhler K, Siddiqi I, Vasef MA. (2018) BRAF V600E mutation across multiple tumor types: correlation between

DNA-based sequencing and mutation-specific immunohistochemistry. Appl Immunohistochem Mol Morphol 26(10):709–713. https://doi.org/10.1097/PAI.0000000000000516.
27. Martinuzzi C, Pastorino L, Andreotti V, Garuti A, Minuto M, Fiocca R, Bianchi-Scarrà G, Ghiorzo P, Grillo F, Mastracci L. (2016) A combination of immunohistochemistry and molecular approaches improves highly sensitive detection of BRAF mutations in papillary thyroid cancer. Endocrine 53(3):672–680. https://doi.org/10.1007/s12020-015-0720-9.
28. McKelvie PA, Chan F, Yu Y, Waring P, Gresshoff I, Farrell S, Williams RA. The prognostic significance of the BRAF V600E mutation in papillary thyroid carcinoma detected by mutation-specific immunohistochemistry. (2013) Pathology 45(7):637–644. https://doi.org/10.1097/PAT.0000000000000008.
29. Na JI, Kim JH, Kim HJ, Kim HK, Moon KS, Lee JS, Lee JH, Lee KH, Park JT. (2015) VE1 immunohistochemical detection of the BRAF V600E mutation in thyroid carcinoma: a review of its usefulness and limitations. Virchows Arch 467(2):155–168. https://doi.org/10.1007/s00428-015-1773-0.
30. Oh HS, Kwon H, Park S, Kim M, Jeon MJ, Kim TY, Shong YK, Kim WB, Choi J, Kim WG, Song DE. (2018) Comparison of immunohistochemistry and direct sanger sequencing for detection of the BRAF(V600E) mutation in thyroid neoplasm. Endocrinol Metab (Seoul) 33(1):62–69. https://doi.org/10.3803/EnM.2018.33.1.62.
31. Paja Fano M, Ugalde Olano A, Fuertes Thomas E, Oleaga Alday A. Immunohistochemical detection of the BRAF V600E mutation in papillary thyroid carcinoma. Evaluation against real-time polymerase chain reaction. (2017) Endocrinol Diabetes Nutr 64(2):75–81. https://doi.org/10.1016/j.endinu.2016.12.004.
32. Qiu T, Lu H, Guo L, Huang W, Ling Y, Shan L, Li W, Ying J, Lv N. (2015) Detection of BRAF mutation in Chinese tumor patients using a highly sensitive antibody immunohistochemistry assay. Sci Rep 5:9211. https://doi.org/10.1038/srep09211.
33. Routhier CA, Mochel MC, Lynch K, Dias-Santagata D, Louis DN, Hoang MP. (2013) Comparison of 2 monoclonal antibodies for immunohistochemical detection of BRAF V600E mutation in malignant melanoma, pulmonary carcinoma, gastrointestinal carcinoma, thyroid carcinoma, and gliomas. Hum Pathol 44(11):2563–2570. https://doi.org/10.1016/j.humpath.2013.06.018.
34. Rushton S, Burghel G, Wallace A, Nonaka D. (2016) Immunohistochemical detection of BRAF V600E mutation status in anaplastic thyroid carcinoma. Histopathology 69(3):524–526. https://doi.org/10.1111/his.12964.
35. Sun J, Zhang J, Lu J, Gao J, Lu T, Ren X, Duan H, Liang Z. (2015) Immunohistochemistry is highly sensitive and specific for detecting the BRAF V600E mutation in papillary thyroid carcinoma. Int J Clin Exp Pathol 8(11):15072–15078.
36. Szymonek M, Kowalik A, Kopczyński J, Gąsior-Perczak D, Pałyga I, Walczyk A, Gadawska-Juszczyk K, Płusa A, Mężyk R, Chrapek M, Góźdź S, Kowalska A. (2017) Immunohistochemistry cannot replace DNA analysis for evaluation of BRAF V600E mutations in papillary thyroid carcinoma. Oncotarget 8(43):74897–74909. https://doi.org/10.18632/oncotarget.20451.
37. Takada N, Mussazhanova Z, Hirokawa M, Nakashima M, Miyauchi A. (2018) Immunohistochemical and molecular analyses focusing on mesenchymal cells in papillary thyroid carcinoma with desmoid-type fibromatosis. Pathobiology 85(5–6):300–303. https://doi.org/10.1159/000492117.
38. Zagzag J, Pollack A, Dultz L, Dhar S, Ogilvie JB, Heller KS, Deng FM, Patel KN. (2013) Clinical utility of immunohistochemistry for the detection of the BRAF v600e mutation in papillary thyroid carcinoma. Surgery 154(6):1199–1204. https://doi.org/10.1016/j.surg.2013.06.020.
39. Zhang X, Wang L, Wang J, Zhao H, Wu J, Liu S, Zhang L, Li Y, Xing X. (2018) Immunohistochemistry is a feasible method to screen BRAF V600E mutation in colorectal and papillary thyroid carcinoma. Exp Mol Pathol 105(1):153–159. https://doi.org/10.1016/j.yexmp.2018.07.006.
40. Zhu X, Luo Y, Bai Q, Lu Y, Lu Y, Wu L, Zhou X. (2016) Specific immunohistochemical detection of the BRAF V600E mutation in primary and metastatic papillary thyroid carcinoma. Exp Mol Pathol 100(1):236–241.https://doi.org/10.1016/j.yexmp.2016.01.004.
41. Pyo JS, Sohn JH, Kang G. (2015). BRAF immunohistochemistry using clone VE1 is strongly concordant with BRAF(V600E) mutation test in papillary thyroid carcinoma. Endocr Pathol 26(3):211–217. https://doi.org/10.1007/s12022-015-9374-7.

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