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# BRAF V600E Mutation as a Predictor of Thyroid Malignancy in Indeterminate Nodules

## A Systematic Review and Meta-analysis

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## ABSTRACT

**Background:** Thyroid nodules are usually diagnosed using fine-needle aspiration (FNA). The sensitivity limitations of FNA result in 10-30% of nodules being classified as "indeterminate". The $BRAF^{V600E}$ mutation is associated with papillary thyroid carcinoma (PTC). We conducted a systemic review and meta-analysis to evaluate the diagnostic utility of the $BRAF^{V600E}$ mutation in indeterminate nodules.

**Method:** PUBMED and EMBASE were searched for studies testing for the $BRAF^{V600E}$ involving indeterminate nodules (Thy3a, Thy3f, Thy4) and containing information on final surgical histopathology. Thirty two studies involving 3,150 indeterminate nodules were included in the analysis.

**Results:** The overall sensitivity and specificity for $BRAF^{V600E}$ for the diagnosis of thyroid malignancy was 0.40 (95% CI:0.32–0.48) and 1.00 (95% CI:0.98–1.00) respectively. The diagnostic odds ratio (DOR) was 205.4 (95% CI:40.1-1052). With a Fagan plot, the post-test probability of thyroid cancer, given a negative mutation was 6%, but this rose to 92% with a positive result. On subgroup analysis, for Thy3a nodules, the pooled sensitivity and specificity for thyroid malignancy was 0.21 (95% CI:0.13-0.34) and 1.00 (95% CI:0.98-1.00). For Thy3f nodules, the pooled sensitivity and specificity was 0.09 (95% CI:0.03-0.20) and 1.00 (95% CI:0.05-1.00) respectively. For Thy4 nodules, the corresponding sensitivity and specificity was 0.58 (95%CI:0.5-0.64) and 0.99 (95%CI:0.95-1.00) respectively.

**Conclusions:** Despite a high specificity for thyroid cancer, $BRAF^{V600E}$ mutation has a low overall sensitivity and therefore has a limited diagnostic value as a single screening test.

## INTRODUCTION

Thyroid cancer is the most common endocrine malignancy with increasing incidence worldwide.[1] Recent data indicate that thyroid cancer is currently the fifth commonest cancer in the world, representing about 6% of all cancer incidence in women.[2,3] Ultrasound-guided fine needle aspiration (US-FNA) is a crucial diagnostic tool for the identification of nodules harbouring malignancy.[4] FNA is accurate, safe, cost-effective, and a minimally invasive procedure in the diagnosis of thyroid nodules.[5] However, despite its high sensitivity, specificity and accuracy, FNA is limited by inadequate/insufficient samples (10-20%) or indeterminate cytological results (10-30%).[4,6] Within the indeterminate category (Thy3a, Thy3f and Thy4), the risk of malignancy varies from between 5% and 75% depending on the different cytological classification.[7] Within the category "atypia of undetermined significance" (AUS) or "follicular lesion of undetermined significance" (FLUS), the recommended management is clinical correlation and a repeat FNA at an appropriate time interval.[8] However, following repeated FNA, 50% of patients will require diagnostic thyroid surgery, as the diagnosis is indeterminate.[9,10] Within the "suspicious for follicular neoplasm" (SFN), "follicular neoplasm" (FN) and "suspicious for malignancy" (SFM) categories, thyroid surgery (thyroid lobectomy or total thyroidectomy) is usually recommended.[4]

With the current clinical algorithm, especially in cases treated non-operatively, a false negative FNA result can lead to a delay in treatment and a less favourable prognosis.[11,12] Furthermore, a significant proportion of patients with indeterminate cytology will undergo diagnostic surgery and of these, only 10-40% turned out to be malignant on final histopathology resulting in unnecessary surgeries with the attendant risks and expenses.[13] Because of the inherent limitation of FNA, many efforts have been directed at improving its diagnostic accuracy especially in the diagnosis of thyroid malignancy.

Since its initial description and association with thyroid cancer,[14,15] the oncogenic $BRAF^{V600E}$ has been extensively studied and its potential in clinical application to diagnose thyroid cancer is increasingly recognized. It is the most common genetic mutation in thyroid cancers, occurring in 35% - 65% of cases of PTC.[14] There is mounting evidence demonstrating that the $BRAF^{V600E}$ mutation may be associated with a poorer prognosis and more aggressive tumour behaviour (extrathyroidal extension, lymph node metastasis and recurrence).[16] Furthermore, $BRAF^{V600E}$ mutation was shown to be an independent prognostic factor for PTC recurrence and associated with increased cancer-related mortality.[17,18]

The aim of this study was to evaluate the sensitivity and specificity of the $BRAF^{V600E}$ mutation in predicting thyroid malignancy in indeterminate nodules. We undertook a systematic review and meta-analysis of the published literature describing the utility of $BRAF^{V600E}$ mutation in diagnosing indeterminate nodules. This updated meta-analysis also compares the diagnostic utility of $BRAF^{V600E}$ in different cytology within the indeterminate group.

## METHODS

### *Search Strategy*

Studies evaluating the utility of $BRAF^{V600E}$ mutation in diagnosing thyroid malignancy in indeterminate nodules were reviewed. We searched PUBMED and EMBASE for English language publications relevant to our topic. Articles were identified using the following search terms: “BRAF”, “B-RAF”, “thyroid”, “indeterminate”, “undetermined”, “nodule”, “cytologically”, “cytology”, “FNA”, “FNAB”, “AUS”, “FLUS”, “FN/SFN” and “SFM”. We utilised the Boolean operator of “OR”, “AND” and “NOT” between the search terms (**Table S1** in the Supplement). We also conducted a manual search for additional relevant studies in

the reference lists of articles retrieved. The guidelines for Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used.[19]

***Inclusion and exclusion criteria***

The following inclusion criteria were used: (1) Pre-operative FNAs identifying indeterminate nodules (2) $BRAF^{V600E}$ mutation tested in these thyroid nodules; (3) The corresponding histopathology following surgical excision was reported; and (4) the sensitivity and specificity of $BRAF^{V600E}$ was reported or could be calculated from the data provided. For papers not acquired or those where data could not be extracted, respective authors were contacted. Reasons for study exclusions are detailed in **Figure 1**. We defined indeterminate nodules as those with no definitive diagnosis of benign, malignant; thus including AUS/ FLUS, FN/SFN, and SFM. This corresponds with Thy3a, Thy3f and Thy4 (Bethesda III-V).[20,21]

***Data extraction and quality assessment***

Three reviewers (MJ, OO, and LH) independently extracted relevant data from each eligible study. The following data were collected: author's name, year of publication, country, reference standard, method of $BRAF^{V600E}$ detection, number of participants and nodules involved, and number of samples with corresponding histopathological results. Disagreements and discordant values were resolved by discussion and joint review by all reviewers. Each eligible study was assessed for quality using the revised Quality Assessment for Studies of Diagnostic Accuracy (QUADAS-2) tools.[22]

***Data Synthesis and Statistical Analysis***

The main outcome parameters were; pooled sensitivity; specificity and positive likelihood ratio (PLR); and the corresponding confidence intervals (CIs) by random effect model. Pooled sensitivity and specificity for $BRAF^{V600E}$ test performance is displayed using a forest

plot. Subgroup analyses were additionally performed according to different Thy classifications and $BRAF^{V600E}$ detection methods. Study heterogeneity was assessed using the Q-test and inconsistency index ($I^2$ statistic). Test performance in the presence of heterogeneity was summarized using hierarchical summary receiver operator curves (HSROC) and area under the curve (AUC) was applied to demonstrate the overall diagnostic performance. To explore the sources of between-study heterogeneity, a meta-regression method of Reitsma et al.[34] was applied to evaluate the effects of covariates. Diagnostic odds ratio (DOR) was also calculated as a single indicator measure of the diagnostic tests accuracy. Deek's Funnel Plot Asymmetry Test was applied to visually determine the presence of publication bias.

## RESULTS

### *Literature Search Outcome*

A total of 522 articles were retrieved; 190 articles were removed as duplicates and the remaining 332 studies screened. Upon initial abstract review, 181 articles were excluded (see **Figure 1**). The commonest exclusion reason was an inappropriate study focus (n=80). One hundred and nineteen articles were excluded upon secondary review. The remaining 32 studies were selected for inclusion because they fulfilled the study criteria. There was 100% agreement between reviewers at the level of study selection from full-text articles (Cohen weighted κ was 1.0; SD=0). Thirty two studies with a total of 3,150 indeterminate thyroid nodules were included in the meta-analysis.[23–54] The main characteristics of the included studies are summarised in **Table S2** in supplementary content. Overall, we observed high study quality across all the included studies (**Figure S2** in supplementary).

***Overall Diagnostic performance***

The pooled sensitivity and specificity of $BRAF^{V600E}$ mutation in the diagnosis of thyroid malignancy was 0.40 (95% CI: 0.32-0.48) and 1.00 (95% CI, 0.98-1.00) respectively (**Figure 2, Table 1**). Overall, $BRAF^{V600E}$ mutation has good diagnostic performance with AUC of 0.87 (**Figure 3**). The pre-test probability of thyroid malignancy in indeterminate nodules was 10% in this meta-analysis and the post-test probability of thyroid malignancy, given a negative mutation detection result, was 6%, but rose to 92% with a positive result (**Figure 4**). Significant heterogeneity was observed ($P < 0.0001$, $I^2 = 89.94\%$ and $P < 0.0001$, $I^2 = 90.01\%$). The Deek's funnel plot revealed an asymmetry test with $P < 0.0001$ for the slope coefficient, demonstrating a publication bias.

***Thy3a***

For Thy3a category, the pooled sensitivity and specificity of $BRAF^{V600E}$ mutation in the diagnosis of thyroid malignancy was 0.21 (95% CI: 0.13 - 0.34) and 1.00 (95% CI: 0.98 - 1.00) respectively, with significant heterogeneity ($P < 0.0001$, $I^2 = 91.96\%$ and $P < 0.0001$, $I^2 = 89.51\%$) (**Table 1**). The AUC was 0.85. The DOR was 163 (95% CI: 11.2 – 2368). A Fagan plot revealed that with a pre-test probability of thyroid malignancy in indeterminate nodules of 10%, the post-test probability of thyroid malignancy, given a negative mutation detection result, was 8%, but 89% with a positive result.

***Thy3f***

For Thy3f, the pooled sensitivity and specificity of $BRAF^{V600E}$ mutation in the diagnosis of thyroid malignancy was 0.09 (95% confidence interval: 0.03 - 0.20) and 1.00 (95% CI: 0.05 - 1.00) respectively (See **Table 1**). The AUC was 0.77. A Fagan plot revealed that the post-test probability of thyroid malignancy, given a negative mutation detection result was 9% and 100% with a positive result.

### *Thy4*

For Thy4, the pooled sensitivity and specificity was 0.58 (95% CI: 0.5 - 0.64) and 0.99 (95% CI: 0.95 - 1.00) respectively (See **Table 1**). The AUC was 0.87. The Fagan plot, demonstrated that the post-test probability of thyroid malignancy, given a negative mutation detection result, was 5%, and 90% with a positive result.

### *Subgroup analysis according to BRAF$^{V600E}$ detection methods*

A subgroup analysis was performed to determine whether there was any fundamental difference in sensitivity and specificity based on the methods by which BRAF$^{V600E}$ is detected. Overall, there was a significant difference in the ability of each method to detect BRAF$^{V600E}$ mutation. Both Sanger sequencing (n=15) and real time PCR (n=10) achieved 100% specificity with false negative rates of 26.8% and 23.6% respectively. Pyrosequencing method (n=4) resulted in 97% specificity with comparable false negative rates of 24.4%. Immunohistochemistry method (n=3) resulted in a much lower specificity of 91% and a much higher false negative rate of 38.9% in this cohort (See **Table 1**).

### *Histopathology*

Among the 3,150 indeterminate thyroid nodules, 670 were positive for the BRAF$^{V600E}$ mutation (21.3%). Of the 1,487 thyroid cancer, 662 (44.5%) tested positive for BRAF$^{V600E}$ mutation. The various histologic subtypes are summarized in the supplementary content. PTC and its histologic subtypes were the most common malignancy representing 99.8% of all malignancy in this group (n = 661). The most common histological subtype was classical PTC (n= 638), followed by follicular variant PTC (n = 21) and tall cell PTC (n = 2). Only 1 nodule with the BRAF$^{V600E}$ mutation was reported as a Hürthle cell carcinoma, a variant of follicular neoplasm. Eight nodules with a BRAF$^{V600E}$ mutation were histologically benign (false positive). The prevalence of BRAF$^{V600E}$ mutation varies according to different cytological groups and populations studied. The prevalence rate of BRAF$^{V600E}$ mutation in

indeterminate nodules was 21.3%. For Thy3a, Thy3f and Thy4, the prevalence rate was 12.8%, 3.6% and 49.7% respectively.

## DISCUSSION

Incidental findings of thyroid nodules are becoming more common. Being able to accurately distinguish between those that require surgery and those that do not is a significant challenge, with an indeterminate result occurring in approximately 10%-30% of thyroid FNAs.[6] Some patients, following consultation with their surgeons opt for an operative approach. Nonetheless, 60% of indeterminate nodules are benign on final pathological analysis; resulting in unnecessary surgery in a majority of patients.[8] AUS/FLUS on a single FNAC is associated with a finding of malignancy on final pathology in between 15% and 30% of patients. In those with two FNAC results showing AUS/FLUS the risk of malignancy rises to 25%.[7]

The association of $BRAF^{V600E}$ mutation with PTC was initially suggested by Cohen *et al* in 2003.[14] The application of $BRAF^{V600E}$ mutation as a preoperative diagnosis tool for PTC was suggested by Xing et al in 2004.[55] Since then, it has been extensively studied and available data favours its clinical use as an adjunct to FNA in the preoperative diagnosis of thyroid malignancy. Despite its high specificity, $BRAF^{V600E}$ mutation alone is unlikely to provide a full picture of thyroid carcinogenesis. Other genetic mutations that have been implicated in thyroid carcinoma include RET-PTC, PAX8-PPARγ rearrangements and RAS point mutations.[37] In fact, several panels testing for these common genes including $BRAF^{V600E}$ mutation are undergoing evaluation for clinical application. The Quest Diagnostics Thyroid Cancer Mutation Panel which incorporates the above genes demonstrates a PPV of 88%, 87% and 95% respectively for AUS/FLUS, FN and SFM.[33] In our study we found that $BRAF^{V600E}$ mutation alone demonstrated a PPV of 96.9%, 95.4% and 99.8% respectively for the same

categories. Although this seems counter-intuitive, the most likely explanation for this is probably because the study by Nikiforov *et al* included a relatively smaller number of patients in a population where the prevalence of $BRAF^{V600E}$ mutation is possibly low. However, it is worth mentioning that a thyroid nodule tested positive for any of these genes ($BRAF^{V600E}$, RET-PTC or PAX8-PPARγ) was associated with 100% risk of cancer.[34]

Furthermore, because $BRAF^{V600E}$ mutation occurs only in 35-65% of cases of PTC, and has not been found in follicular carcinoma,[55] its clinical application as a sole marker in preoperative thyroid cancer diagnosis remains limited. In our study, we found that only 44.5% of thyroid cancer tested positive for $BRAF^{V600E}$ mutation. Therefore, a question remains on the indeterminate thyroid nodules tested negative for $BRAF^{V600E}$ mutation in which malignancy cannot be ruled out. In this case, several alternatives are feasible such as the utility of molecular profiling tests. Veracyte's gene-expression classifier (GEC) for example incorporates 167 genes to classify thyroid nodules as either benign or suspicious and has a sensitivity and specificity of 92% and 52% respectively.[56] In addition, Keutgen *et al* demonstrated that a panel of 4-micro RNAs (MiR-222, miR-328, miR-197 and miR-21) in a preliminary setting correctly identified benign and malignant indeterminate nodules with 100% sensitivity and 86% specificity.[57] These panels of molecular tests provide an excellent assessment for indeterminate thyroid nodules with increasing use in clinical setting. Despite this, their use widespread use may be limited by the cost incurred in each sample tested. The commercial cost of the Quest Diagnostics Thyroid Cancer Mutation Panel for example is about $3000 per sample while that of Veracyte's GEC is about $3,200 per sample.[58] In comparison, $BRAF^{V600E}$ testing is already quite established in clinical setting and costs approximately $500 - $600 between different institutions. Therefore, it could be argued that $BRAF^{V600E}$ mutation testing offers a more cost-effective approach as a first molecular test for

patients with indeterminate nodules especially in areas where sophisticated molecular testing is not available.

The limitations observed in this meta-analysis are those common to many meta-analyses: namely publication bias; selection bias; lack of complete datasets from individual studies and between-study heterogeneity. Publication bias was observed in our meta-analysis. In our search strategy, we attempted to include as many key words and relevant works as possible, although we acknowledge that this review may not be exhaustive. We searched only PUBMED and EMBASE as we believed that these two databases represent the majority of candidate papers, although this may have resulted in "missing papers". Despite these concerns, we believe that the papers included in our review account for the vast majority of all the papers relevant to the topic and were otherwise representative.

Heterogeneity between studies was also observed, and may represent a further potential source of bias. Study heterogeneity is pervasive in meta-analyses, and in this meta-analysis it is contributed to mainly by variations in study design, patient selection and demography, clinical setting, $BRAF^{V600E}$ detection methods, the type of reference standards, or a combination of these factors. Design flaws within the studies enrolled may also contributed to the heterogeneity. We controlled for between-study heterogeneity using the random-effects regression model, taking into account a number of clinically important covariates. Nevertheless, we appreciate that residual confounders may still be present.

## CONCLUSION

While the authors accept that some of the limitations of the study include false negative and false positive rates, used in the correct clinical setting $BRAF^{V600E}$ testing could aid

stratification of indeterminate nodules into a more high risk category, and therefore one that warrants surgical excision. In areas where molecular profiling tests are not routinely performed, $BRAF^{V600E}$ mutation could be tested as an adjunct to FNAs in indeterminate thyroid nodules. Our analysis supports a decision that all patients with a $BRAF^{V600E}$-positive FNA should be offered the choice of total thyroidectomy because of the high risk of malignancy (98.8% in the cohort reviewed herein) and its association with poor prognosis. Despite this, the value of $BRAF^{V600E}$ mutation as a single screening test for patients with indeterminate nodules is limited due to its low sensitivity. Future direction of research may include assessment on its clinical integration in thyroid nodule assessment in general.

## CONFLICT OF INTEREST STATEMENT

All authors have read the manuscript and declared that there are no conflicts of interests.

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## FIGURES AND TABLES

**Figure 1.** Literature Search of Eligible Studies (Flow chart)

**Figure 2.** Forest plot for sensitivity and specificity of $BRAF^{V600E}$ mutation in diagnosing thyroid malignancy in indeterminate nodules

**Figure 3.** HSROC curve

**Figure 4.** Fagan's nomogram to evaluate the clinical utility of $BRAF^{V600E}$ mutation

**Table 1.** Pooled results of meta-analysis

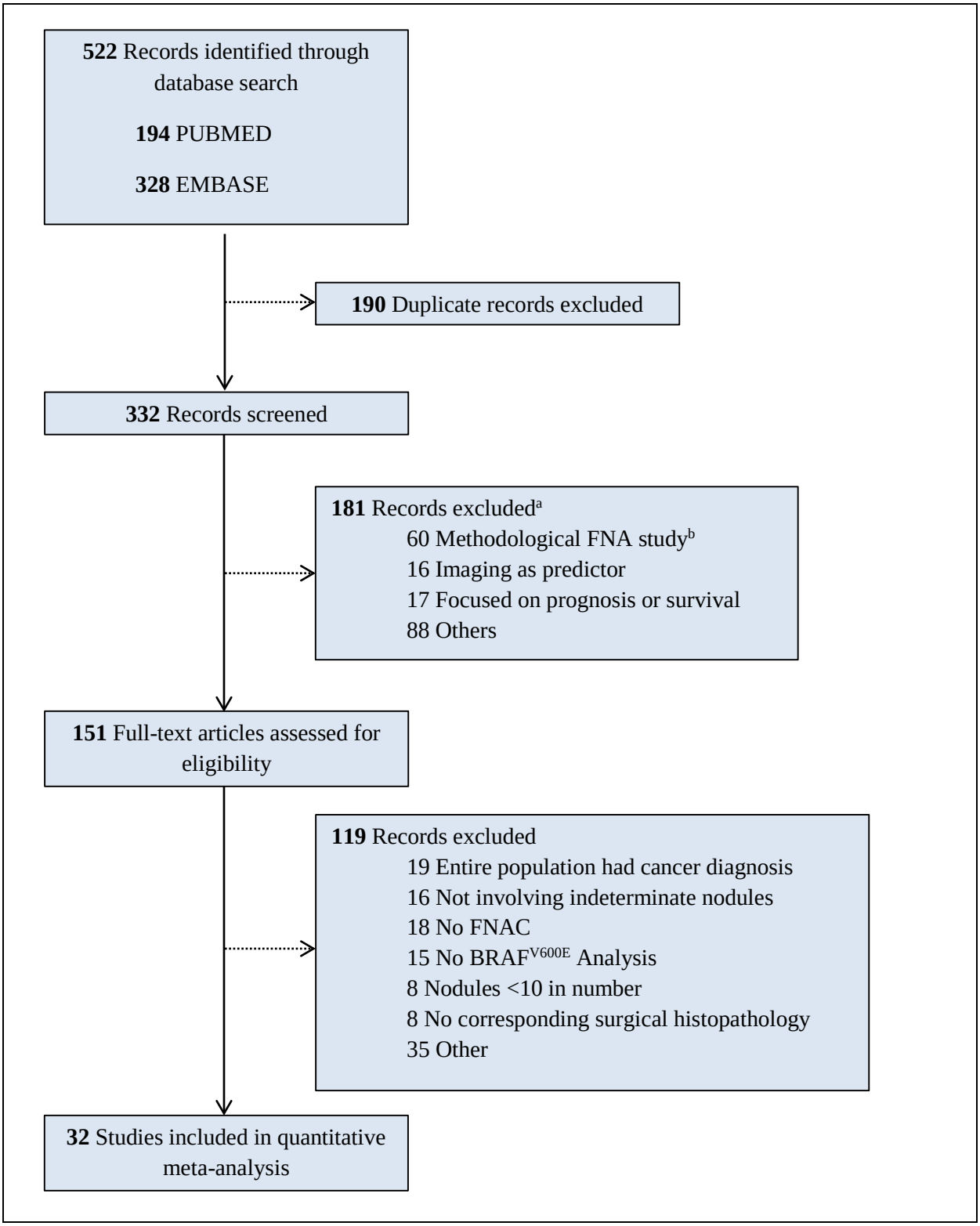



**Figure 1.** Literature Search of Eligible studies

[a] The same study could be excluded for multiple reasons

[b] Focused on techniques and methods on performing FNAC

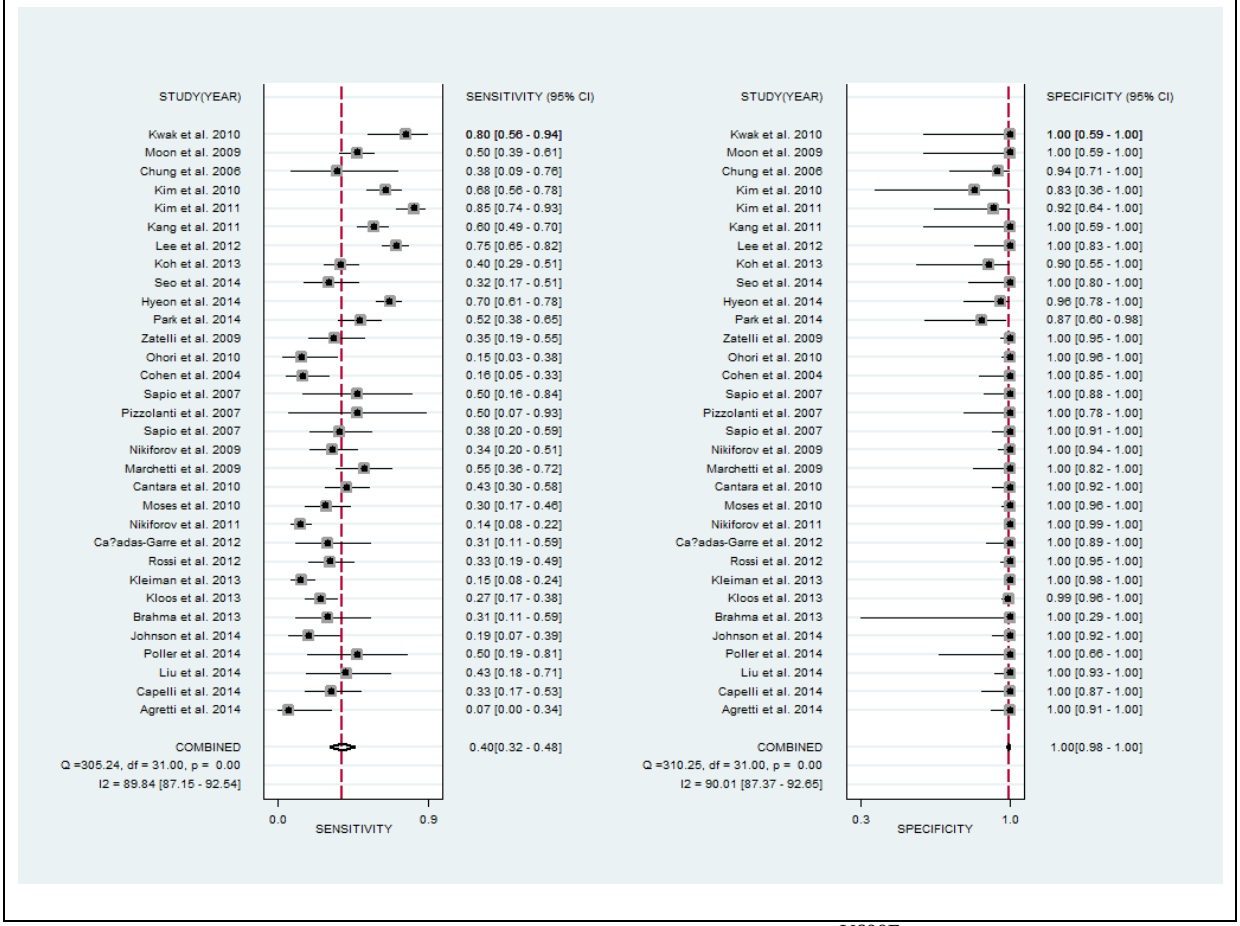



**Figure 2.** Forest plot for the sensitivity and specificity of $BRAF^{V600E}$ mutation in diagnosing thyroid malignancy in indeterminate nodules

The pooled sensitivity was 0.40 (95% CI: 0.32-0.48) and the pooled specificity was 1.00 (95% CI: 0.98-1.00).

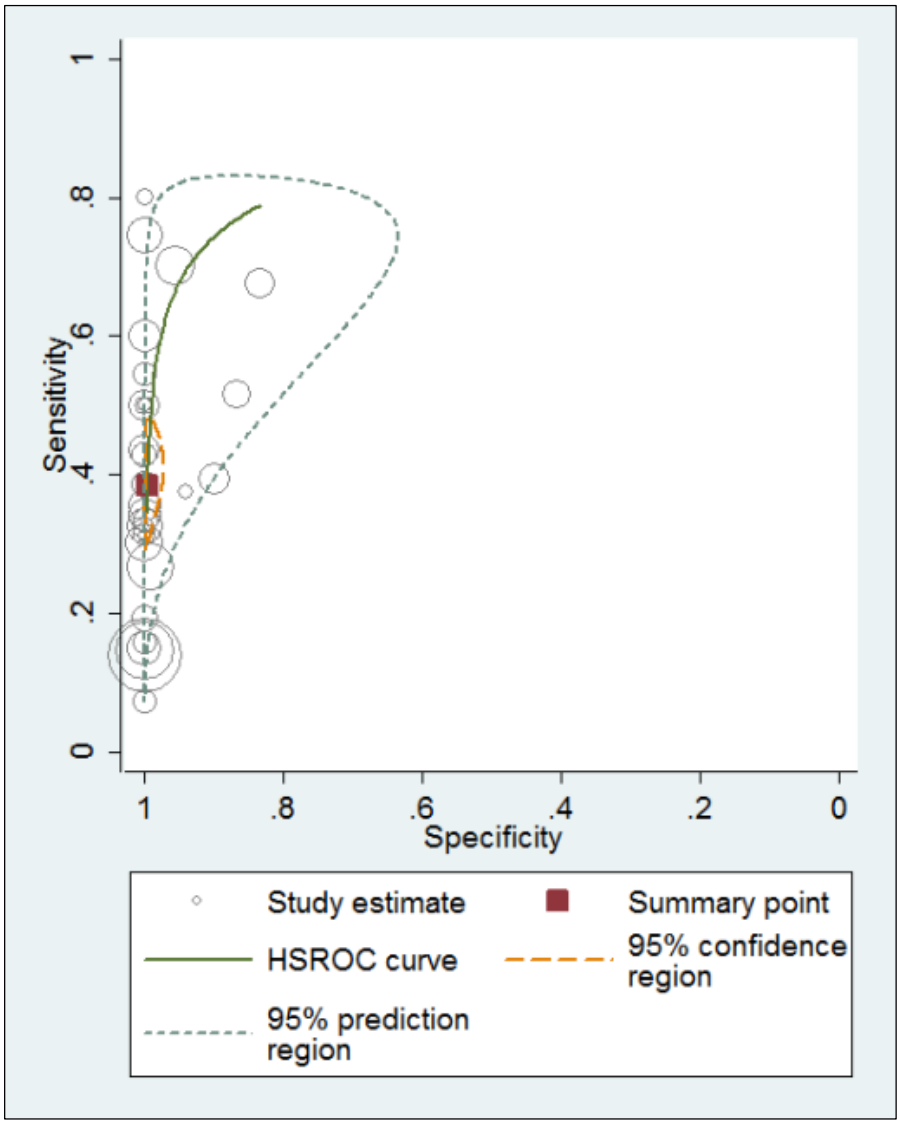



**Figure 3.** HSROC curve

The HSROC curve shows the 95% confidence and prediction regions around mean operating sensitivity and specificity point after outlier is excluded. Area under the curve (AUC) is 0.87.

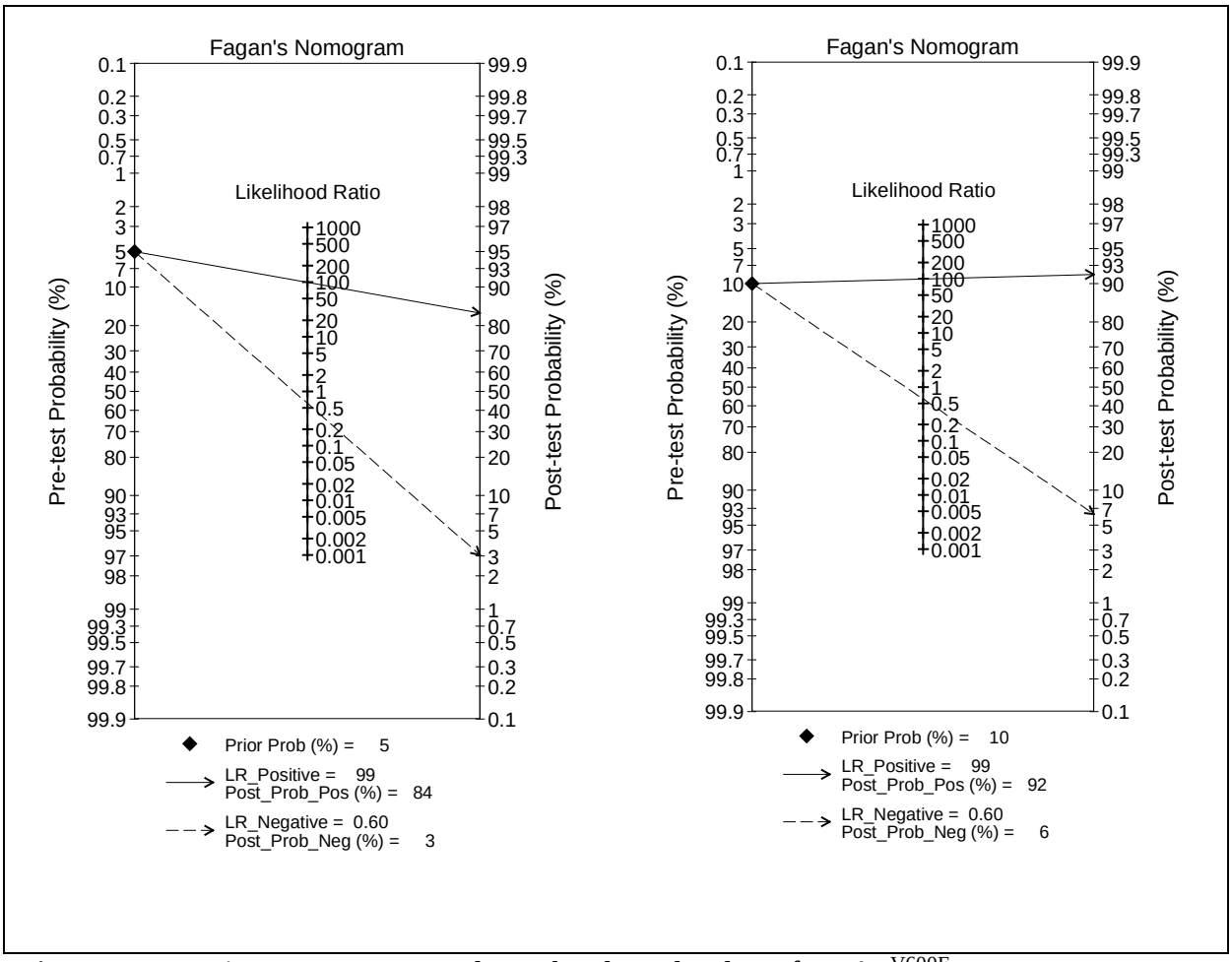



**Figure 4.** Fagan's nomogram to evaluate the clinical utility of $BRAF^{V600E}$ mutation

The Fagan plot showed a pre-test probability of 10% to develop a thyroid malignancy in indeterminate nodules. The post-test probability of thyroid malignancy given a negative $BRAF^{V600E}$ mutation was 6%; and 92% with a positive result.

| Variables | Sensitivity (95% CI) | Specificity (95% CI) | Likelihood ratio | DOR (95% CI) |
|---|---|---|---|---|
| All indeterminate nodules | 0.40 (0.32-0.48) | 1.00 (0.98-1.00) | 98.7 | 164.0 |
| Thy3a nodules | 0.21 (0.13-0.34) | 1.00 (0.98-1.00) | 76.7 | 97.1 |
| Thy3f nodules | 0.09 (0.03-0.20) | 1.00 (0.98-1.00) | 23539 | 25068 |
| Thy4 nodules | 0.58 (0.50-0.64) | 0.99 (0.95-1.00) | 79.2 | 185.1 |
| **BRAF detection methods** | | | | |
| PCR Sanger sequencing (n=15) | 0.45 (0.42-0.49) | 1.00 (0.99-1.00) | 172.2 | 314.7 |
| Real time PCR (n=10) | 0.32 (0.28-0.37) | 1.00 (0.99-1.00) | 249.7 | 366.6 |
| Pyrosequencing (n=4) | 0.60 (0.52-0.68) | 0.97 (0.92-0.99) | 20.6 | 50.26 |
| Immunohistochemistry (n=3) | 0.56 (0.48-0.63) | 0.91 (0.72-0.99) | 6.4 | 13.36 |

**Table 1.** Pooled results of the meta-analysis of the diagnostic accuracy of BRAF V600E mutation detection in thyroid malignancy

*DOR*= diagnostic odds ratio.

## SUPPLEMENTARY FIGURES AND TABLES

**Table S1.** Literature search algorithm

**Table S2.** Participants and study characteristics

**Figure S1.** Summary of study quality according to QUADAS-2

**Figure S2.** Deek's funnel plot with superimposed regression line to determine publication bias.

| No | Search terms | PUBMED | EMBASE | Search results |
|---|---|---|---|---|
| **#1** | BRAF OR B-RAF | 7343 | 15,042 | **22,385** |
| **#2** | thyroid | 174,765 | 214,948 | **389,713** |
| **#3** | Nodule OR undetermined OR indeterminate | 374,033 | 84,434 | **458,467** |
| **#4** | cytologically OR cytology OR FNA OR FNAB OR AUS OR FLUS OR FN/SFN OR SFM | 226,456 | 167,998 | **394,454** |
| **#5** | #1 AND #2 AND #3 AND English[la] | 239 | 407 | **646** |
| **#6** | #4 AND case reports[pt] | 0 | 48 | **48** |
| **#7** | #4 AND letter[pt] | 4 | 3 | **7** |
| **#8** | #4 AND review[pt] | 24 | 74 | **98** |
| **#9** | #4 AND editorial[pt] | 1 | 11 | **12** |
| **#10** | #4 AND practice guideline[pt] | 0 | 8 | **8** |
| **#11** | #4 AND historical article[pt] | 0 | 0 | **0** |
| **#12** | #4 AND news[pt] | 1 | 0 | **1** |
| **#13** | #4 AND meta-analysis[pt] | 1 | 6 | **7** |
| **#14** | #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 | 30 | 94 | **124** |
| **#15** | #5 NOT #14 | 149 | 309 | **522** |

**Table S1.** Literature search algorithm

Key. [la] language, [pt] publication type

| Study | Country | Study Method | BRAF detection method | FNA Reporting | Total nodules | TP | FP | FN | TN | BRAF incidence |
|---|---|---|---|---|---|---|---|---|---|---|
| Agretti et al [35] | Italy | Prospective | PCR Sanger Sequencing | NCI | 54 | 1 | 0 | 13 | 40 | 1.8% |
| Capelli et al [36] | Italy | Prospective | PCR Pyrosequencing | BTA | 56 | 10 | 0 | 20 | 26 | 17.9% |
| Liu et al [37] | China | Prospective | PCR Pyrosequencing | BSRTC | 63 | 6 | 0 | 8 | 49 | 9.5% |
| Poller et al [38] | UK | Prospective | Real-time PCR | BTA | 19 | 5 | 0 | 5 | 9 | 26.3% |
| Johnson et al [39] | UK | Prospective | Real-time PCR | BTA | 68 | 5 | 0 | 21 | 42 | 7.4% |
| Brahma et al [40] | Indonesia | Prospective | PCR Sanger Sequencing | NR | 19 | 5 | 0 | 11 | 3 | 26.3% |
| Kloos et al [41] | USA | Prospective | Real-time PCR | BSRTC | 208 | 20 | 1 | 55 | 132 | 10.1% |
| Kleiman et al [42] | USA | Retrospective | PCR Sanger sequencing | BSRTC | 310 | 13 | 0 | 76 | 221 | 4.2% |
| Rossi et al [43] | Italy | Prospective | PCR Sanger sequencing | NCI | 123 | 14 | 0 | 29 | 80 | 11.4% |
| Cañadas-Garre et al [44] | Spain | Prospective | PCR Sanger sequencing | BSRTC | 45 | 5 | 0 | 11 | 31 | 10.6% |
| Nikiforov et al [45] | USA | Prospective | Real-time PCR | BSRTC | 513 | 17 | 0 | 104 | 392 | 3.3% |
| Moses et al [46] | USA | Prospective | PCR Sanger Sequencing | NCI | 137 | 13 | 0 | 30 | 94 | 9.5% |
| Cantara et al [47] | Italy | Prospective | PCR Sanger Sequencing | NR | 95 | 23 | 0 | 30 | 42 | 24.2% |
| Marchetti et al [48] | Italy | Retrospective | PCR Sanger Sequencing | BTA | 52 | 18 | 0 | 15 | 19 | 34.6% |
| Nikiforov et al [49] | USA | Prospective | PCR Sanger Sequencing | NCI | 103 | 14 | 0 | 27 | 62 | 13.6% |
| Sapio et al [50] | Italy | Prospective | Real-time PCR | NCI | 67 | 10 | 0 | 16 | 41 | 14.9% |
| Pizzolanti et al [51] | Italy | Prospective | Real-time PCR | NR | 19 | 2 | 0 | 2 | 15 | 10.5% |
| Sapio et al [52] | Italy | Prospective | Real-time PCR | NCI | 36 | 4 | 0 | 4 | 28 | 11.1% |
| Cohen et al [53] | USA | Retrospective | PCR Sanger Sequencing | NR | 55 | 5 | 0 | 27 | 23 | 9.1% |
| Ohori et al [54] | USA | Retrospective | Real-time PCR | BSRTC | 117 | 3 | 0 | 17 | 97 | 2.6% |
| Zatelli et al [55] | Italy | Prospective | PCR Sanger Sequencing | NCI | 107 | 11 | 0 | 20 | 76 | 10.3% |
| Park et al [56] | Korea | Retrospective | PCR Pyrosequencing | BSRTC | 73 | 30 | 2 | 28 | 13 | 42.5% |
| Hyeon et al [57] | Korea | Retrospective | PCR Sanger Sequencing | BSRTC | 147 | 87 | 1 | 37 | 22 | 59.9% |
| Seo et al [58] | Korea | Retrospective | Real-time PCR | BSRTC | 48 | 10 | 0 | 21 | 17 | 26.3% |
| Koh et al [59] | Korea | Retrospective | Immunohistochemistry | BSRTC | 91 | 32 | 1 | 49 | 9 | 36.3% |
| Lee et al [60] | Korea | Prospective | PCR Sanger sequencing | NCI | 126 | 79 | 0 | 27 | 20 | 62.7% |
| Kang et al [61] | Korea | Retrospective | Real-time PCR | BSRTC | 102 | 57 | 0 | 38 | 7 | 55.9% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Kim et al [62] | Korea | Prospective | PCR Pyrosequencing | NCI | 74 | 52 | 1 | 9 | 12 | 71.6% |
| Kim et al [63] | Korea | Prospective | Immunohistochemistry | NR | 80 | 50 | 1 | 24 | 5 | 63.8% |
| Chung et al [64] | Korea | Retrospective | PCR Sanger Sequencing | NR | 25 | 3 | 1 | 5 | 16 | 16.0% |
| Moon et al [65] | Korea | Retrospective | PCR Sanger Sequencing | NR | 91 | 42 | 0 | 42 | 7 | 46.2% |
| Kwak et al [66] | Korea | Retrospective | Immunohistochemistry | NCI | 27 | 16 | 0 | 4 | 7 | 59.3% |
| | | | | **TOTAL** | **3150** | **662** | **8** | **825** | **1657** | |

**Table S2.** Participants and study characteristics

NR = Not reported; NCI = National Cancer Institute; BTA = British Thyroid Association; BSRTC = Bethesda system for reporting thyroid cytology

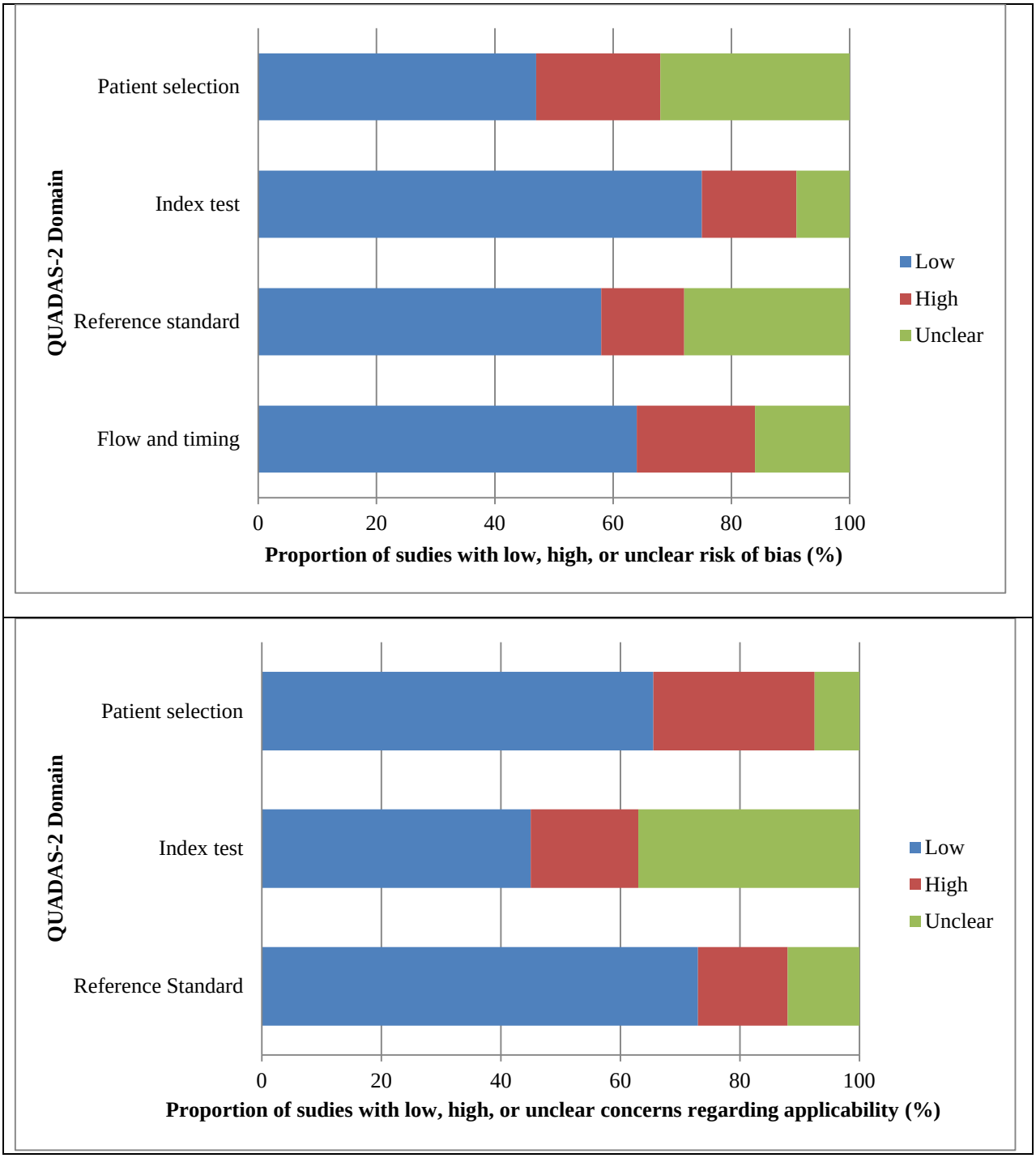



**Figure S1.** Summary of study quality according to QUADAS-2

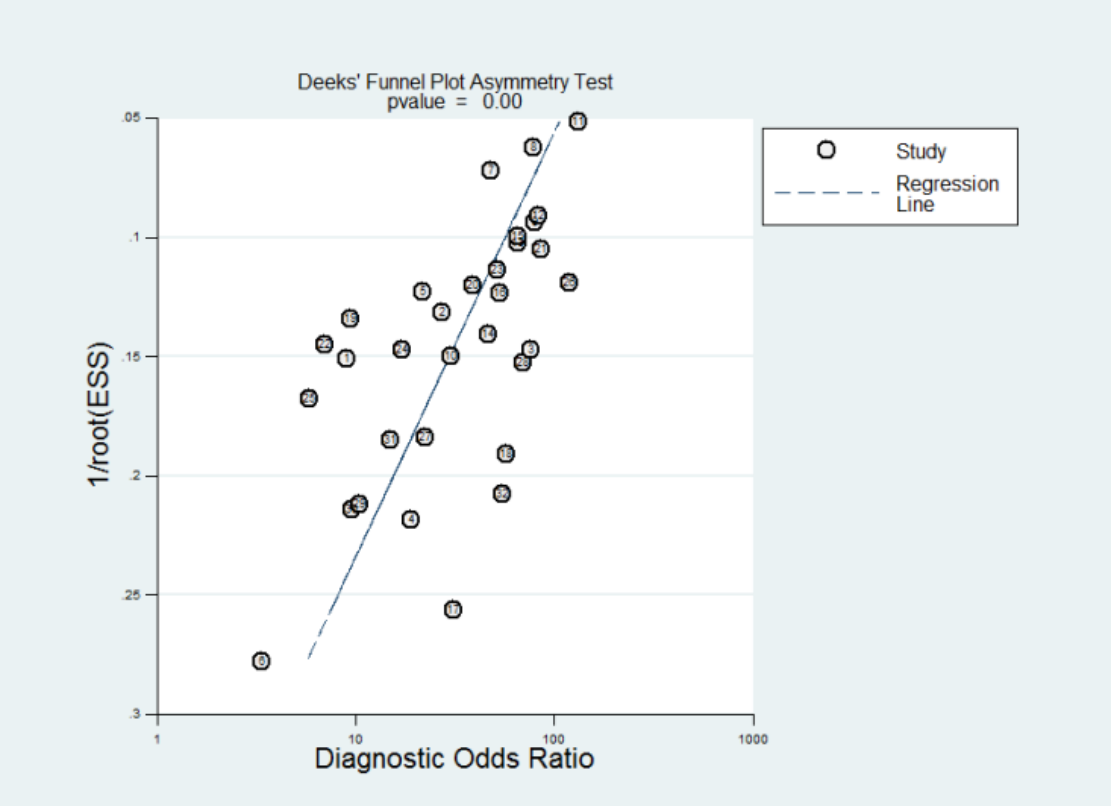



**Figure S2.** Deek's funnel plot with superimposed regression line to determine publication bias.

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