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Title: Molecular Markers in Fine Needle Aspiration of the Thyroid

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Populations	Interventions	Comparators	Outcomes
Individuals: • With thyroid nodule(s) and indeterminate findings on fine needle aspirate	Interventions of interest are: • Fine needle aspirate sample testing with molecular tests to rule out malignancy and to avoid surgical biopsy or resection	Comparators of interest are: • Surgical biopsy	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity • Morbid events • Resource utilization
Individuals: • With thyroid nodule(s) and indeterminate	Interventions of interest are: • Fine needle aspirate sample testing with molecular tests to rule in	Comparators of interest are: • Surgical management based on	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity

Populations	Interventions	Comparators	Outcomes
findings on fine needle aspirate	malignancy and to guide surgical planning	clinicopathologic risk factors	• Morbid events • Resource utilization
Individuals: • With thyroid nodule(s) and indeterminate findings on fine needle aspirate	Interventions of interest are: • Fine needle aspirate sample testing with molecular tests to rule out or to rule in malignancy for surgical planning	Comparators of interest are: • Surgical management based on clinicopathologic risk factors and/or surgical biopsy	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity • Morbid events • Resource utilization

DESCRIPTION

To determine which individuals, need thyroid resection, many physicians will perform a cytologic examination of fine needle aspirate (FNA) samples from a thyroid lesion; however, this method has diagnostic limitations. As a result, assays using molecular markers have been developed to improve the accuracy of thyroid FNA biopsies.

OBJECTIVE

The objective of this evidence review is to evaluate whether testing for molecular markers in fine needle aspirates of the thyroid improves the net health outcome in individuals with thyroid nodule(s) with an indeterminate finding on the fine needle aspirate.

BACKGROUND

Thyroid Nodules

Thyroid nodules are common, present in 5% to 7% of the U.S. adult population; however, most are benign, and most cases of thyroid cancer are curable surgically when detected early.

Diagnosis

Sampling thyroid cells by fine needle aspirate (FNA) is currently the most accurate procedure to distinguish benign thyroid lesions from malignant ones, reducing the rate of unnecessary thyroid surgery for patients with benign nodules and triaging patients with thyroid cancer to appropriate surgery.

About 60% to 70% of thyroid nodules are classified cytologically as benign, and 4% to 10% of nodules are cytologically deemed malignant.[1,] However, the remaining 20% to 30% have equivocal findings, usually due to overlapping cytologic features between benign and malignant nodules; these nodules usually require surgery for a final diagnosis. Thyroid FNA cytology is classified by Bethesda System criteria into the following groups: nondiagnostic; benign; follicular lesion of undetermined significance or atypia of undetermined significance; follicular neoplasm (or suspicious for follicular neoplasm); suspicious for malignancy; and malignant. Lesions with FNA cytology in the atypia of undetermined significance or follicular neoplasm of undetermined significance or follicular neoplasm categories are often considered indeterminate.

Management
There is some individualization of management for patients with FNA-indeterminate nodules, but many patients will require a surgical biopsy, typically thyroid lobectomy, with intraoperative pathology. Consultation would typically be the next step in the diagnosis. Approximately 80% of patients with indeterminate cytology undergo surgical resection; postoperative evaluation has revealed a malignancy rate ranging from 6% to 30%, making this a clinical process with very low specificity.[2,] Thus, if an analysis of FNA samples could reliably identify the risk of malignancy as low, there is potential for patients to avoid surgical biopsy.

Preoperative planning of optimal surgical management in patients with equivocal cytologic results is challenging, because different thyroid malignancies require different surgical procedures (e.g., unilateral lobectomy vs. total or subtotal thyroidectomy with or without lymph node dissection) depending on several factors, including histologic subtype and risk-stratification strategies (tumor size, patient age). If a diagnosis cannot be made intraoperatively, a lobectomy is typically performed, and, if on postoperative histology the lesion is malignant, a second surgical intervention may be necessary for completion of thyroidectomy.

Thyroid Cancer
Most thyroid cancers originate from thyroid follicular cells and include well-differentiated papillary thyroid carcinoma (PTC; 80% of all thyroid cancers) and follicular carcinoma (15%). Poorly differentiated and anaplastic thyroid carcinomas are uncommon and can arise de novo or from preexisting well-differentiated papillary or follicular carcinomas. Medullary thyroid carcinoma originates from parafollicular or C cells and accounts for about 3% of all thyroid cancers.

The diagnosis of malignancy in the case of PTC is primarily based on cytologic features. If FNA in a case of PTC is indeterminate, surgical biopsy with intraoperative pathology consultation is most often diagnostic, although its efficacy and therefore its use will vary across institutions, surgeons, and pathologists. In 2016, reclassification of encapsulated follicular-variant PTC as a noninvasive follicular tumor with papillary-like nuclei was proposed and largely adopted; this classification removes the word *carcinoma* from the diagnosis to acknowledge the indolent behavior of these tumors.[3,]

For follicular carcinoma, the presence of invasion of the tumor capsule or blood vessels is diagnostic, and cannot be determined by cytology, because tissue sampling is necessary to observe these histologic characteristics. Intraoperative diagnosis of follicular carcinoma is challenging and often not feasible because extensive sampling of the tumor and capsule is usually necessary and performed on postoperative, permanent sections.

New approaches for improving the diagnostic accuracy of thyroid FNA include variant analysis for somatic genetic alterations, to more accurately classify which patients need to proceed to surgery (and may include the extent of surgery necessary), and a gene expression classifier to identify patients who do not need surgery and can be safely followed.

Genetic Variants Associated With Thyroid Cancer
A number of genetic variants have been discovered in thyroid cancer. The most common 4 gene variants are *BRAF* and *RAS* single nucleotide variants (SNVs)
and *RET/PTC* and *PAX8/PPARγ* rearrangements.

Papillary carcinomas carry SNVs of the *BRAF* and *RAS* genes, as well as *RET/PTC* and *TRK* rearrangements, all of which can activate the mitogen-activated protein kinase pathway.[4,] These mutually exclusive variants are found in more than 70% of papillary carcinomas. *BRAF* SNVs are highly specific for PTC. Follicular carcinomas harbor either *RAS* SNVs or *PAX8/PPARγ* rearrangements. These variants have been identified in 70% to 75% of follicular carcinomas. Genetic alterations involving the PI3K/AKT signaling pathway also occur in thyroid tumors, although they are rare in well-differentiated thyroid cancers and have a higher prevalence in less differentiated thyroid carcinomas. Additional variants known to occur in poorly differentiated and anaplastic carcinomas involve the *TP53* and *CTNNB1* genes. Medullary carcinomas, which can be familial or sporadic, frequently possess SNVs located in the *RET* gene.

Studies have evaluated the association between various genes and cancer phenotype in individuals with diagnosed thyroid cancer.[5,6,7,]

Telomerase reverse transcriptase (*TERT*) promoter variants occur with varying frequency in different thyroid cancer subtypes. Overall, *TERT* C228T or C250T variants have been reported in approximately 15% of thyroid cancers, with higher rates in the undifferentiated and anaplastic subtypes compared with the well-differentiated subtypes.[8,] *TERT* variants are associated with several demographic and histopathologic features such as older age and advanced TNM stage. *TERT* promoter variants have been reported to be independent predictors of disease recurrence and cancer-related mortality in well-differentiated thyroid cancer.[9,10,11,] Also, the co-occurrence of *BRAF* or *RAS* variants with *TERT* or *TP53* variants may identify a subset of thyroid cancers with unfavorable outcomes.[12,13,14,]

MOLECULAR DIAGNOSTIC TESTING

Variant Detection and Rearrangement Testing

SNVs in specific genes, including *BRAF*, *RAS*, and *RET*, and evaluation for rearrangements associated with thyroid cancers can be accomplished with Sanger sequencing or pyrosequencing or with real-time polymerase chain reaction (PCR) of single or multiple genes or by next-generation sequencing (NGS) panels. Panel tests for genes associated with thyroid cancer, with varying compositions, are also available. For example, Quest Diagnostics offers a Thyroid Cancer Mutation Panel, which includes *BRAF* and *RAS* variant analysis and testing for *RET/PTC* and *PAX8/PPARγ* rearrangements.

The ThyroSeq v3 Next-Generation Sequencing panel (Sonic Healthcare) is an NGS panel of 112 genes. The test is indicated when FNA cytology suggests atypia of uncertain significance or follicular lesion of undetermined significance, follicular neoplasm or suspicious for follicular neoplasm, or suspicious for malignancy.[15,] In particular, it has been evaluated in patients with follicular neoplasm and/or suspicious for follicular neoplasm on FNA as a test to increase both sensitivity and specificity for cancer diagnosis. ThyGenX is an NGS panel that sequences 8 genes and identifies specific gene variants and translocations associated with thyroid cancer. ThyGenX is intended to be used in conjunction with the ThyraMIR microRNA expression test when the initial ThyGenX test is negative.

Gene Expression Profiling

Genetic alterations associated with thyroid cancer can be assessed using gene expression profiling, which refers to the analysis of messenger RNA (mRNA) expression levels of many genes simultaneously. Several gene expression profiling tests are available and stratify tissue from thyroid nodules biologically.

The Afirma Gene Expression Classifier (Afirma GEC; Veracyte) analyzed the expression of 142 different genes to determine patterns associated with benign findings on surgical biopsy. It was designed to evaluate thyroid nodules that have an "indeterminate" classification on FNA as a method to select patients ("rule out") who are at low-risk for cancer. In 2017, Veracyte migrated the Afirma GEC microarray analysis to a next-generation RNA sequencing platform and now markets the Afirma Gene Sequencing Classifier (Afirma GSC) which evaluates 10,196 genes with 1115 core genes.

Other gene expression profiles have been reported in investigational settings but have not been widely validated or used commercially (e.g., Barros-Filho et al [2015],[16,] Zheng et al [2015][17,]); they are not addressed in this review.

ThyraMIR is a microRNA expression-based classifier intended for use in thyroid nodules with indeterminate cytology on FNA following a negative result from the ThyGenX Thyroid Oncogene Panel.

Algorithmic Testing

Algorithmic testing involves the use of 2 or more tests in a prespecified sequence, with a subsequent test automatically obtained depending on results of an earlier test.

Algorithmic Testing Using Afirma GEC With Afirma MTC and Afirma BRAF

In addition to Afirma GSC, Veracyte also markets 2 "malignancy classifiers" that use mRNA expression-based classification to evaluate for *BRAF* variants (Afirma BRAF) or variants associated with medullary thyroid carcinoma (Afirma MTC). Table 1 outlines the testing algorithms for Afirma MTC and Afirma BRAF.

Table 1. Afirma MTC and Afirma BRAF Testing Algorithms

Test 1	Test 1 Result	Reflex to Test 2
Thyroid nodule on fine needle aspirate	"Indeterminate"	Afirma MTC
Afirma GSC	"Malignant" or "suspicious"	Afirma MTC
Afirma GSC	"Suspicious"	Afirma BRAF

Afirma GSC: Afirma Gene Sequencing Classifier; Afirma MTC: Afirma medullary thyroid carcinoma

In a description of the Afirma BRAF test, the following have been proposed as benefits of the mRNA-based expression test for *BRAF* variants: (1) PCR-based methods may have low sensitivity, requiring that a large proportion of the nodule have a relevant variant; (2) testing for only 1 variant may not detect patients with low-frequency variants that result in the same pattern of

pathway activation; and (3) PCR-based approaches with high analytic sensitivity may require a large amount of DNA that is difficult to isolate from small FNA samples.[18,]

The testing strategy for both Afirma MTC and Afirma BRAF is to predict malignancy from an FNA sample with increased pretest probability for malignancy. A positive result with Afirma MTC or Afirma BRAF would inform preoperative planning such as planning for a hemi- versus a total thyroidectomy or performance of central neck dissection.

Algorithmic Testing Using ThyGenX and ThyraMIR
The ThyGenX Thyroid Oncogene Panel (Interpace Diagnostics; testing is done at Asuragen Clinical Laboratory) is an NGS panel designed to assess patients with indeterminate thyroid FNA results. It includes sequencing of 8 genes associated with PTC and follicular carcinomas. ThyGenX has replaced the predicate miR*Inform* Thyroid test that assesses for 17 validated gene alterations.

ThyraMIR (Interpace Diagnostics) is a microRNA expression-based classifier intended for use in thyroid nodules with indeterminate cytology on FNA following a negative result from the ThyGenX Thyroid Oncogene Panel.

The testing strategy for combined ThyGenX and ThyraMIR testing is first to predict malignancy. A positive result on ThyGenX would "rule in" patients for surgical resection. The specific testing results from a ThyGenX positive test would be used to inform preoperative planning when positive. For a ThyGenX negative result, the reflex testing involves the ThyraMIR microRNA expression test to "rule out" for a surgical biopsy procedure given the high negative predictive value of the second test. Patients with a negative result from the ThyraMIR test would be followed with active surveillance and avoid a surgical biopsy.

REGULATORY STATUS
Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Thyroid variant testing and gene expression classifiers are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

In 2013, the THxID™-BRAF kit (bioMérieux), an in vitro diagnostic device, was approved by the U.S. Food and Drug Administration through the premarket approval process to assess specific *BRAF* variants in melanoma tissue via real-time PCR. However, there are currently no diagnostic tests for thyroid cancer mutation analysis with approval from the U.S. Food and Drug Administration. Table 2 provides a summary of commercially available molecular diagnostic tests for indeterminate thyroid pathology.

Table 2. Summary of Molecular Tests for Indeterminate Thyroid Cytopathology FNA Specimens

Test	Predicate	Methodology	Analyte(s)	Report
Afirma® GSC	Afirma®GEC	mRNA gene expression	1115 genes	Benign/suspicious
Afirma® BRAF		mRNA gene expression	1 gene	Negative/positive
Afirma® MTC		mRNA gene expression		Negative/positive
ThyroSeq v3	ThyroSeq v2	Next-generation sequencing	112 genes	Specific gene variant/translocation
ThyGeNEXT®	ThyGenX®[a], miR*Inform*®[a]	Next-generation sequencing	10 genes and 32 gene fusions	Specific gene variant/translocation
ThyraMIR™		microRNA expression	10 microRNAs	Negative/positive
RosettaGX™ Reveal		microRNA expression	24 microRNAs	• Benign • Suspicious for malignancy • High risk for medullary carcinoma

FNA: fine needle aspirate; GEC: Gene Expression Classifier; GSC: Gene Sequencing Classifier; mRNA: messenger RNA; MTC: medullary thyroid carcinoma; PCR: polymerase chain reaction.
[a] The miR*Inform*® test is the predicate test to ThyGenX™ and is not commercially available

POLICY

A. The use of either Afirma® Genomic Sequencing Classifier or ThyroSeq® in fine needle aspirates of thyroid nodules with indeterminate cytologic findings (i.e., Bethesda diagnostic category III [atypia / follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm / suspicion for a follicular neoplasm]) may be considered **medically necessary** in individuals who have **ALL** of the following characteristics:

  1. Thyroid nodules without strong clinical or radiologic findings suggestive of malignancy, **AND**
  2. In whom surgical decision making would be affected by test results.

B. The use of any of the following types of molecular marker testing or gene variant analysis in fine needle aspirates of thyroid nodules with indeterminate findings (Bethesda diagnostic category III [atypia / follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm / suspicion for a follicular neoplasm]) or suspicious findings (Bethesda diagnostic category V [suspicious for malignancy]) to rule in malignancy to guide surgical planning for initial resection rather than a 2-stage surgical biopsy followed by definitive surgery may be considered **medically necessary**:

  1. ThyroSeq®;
  2. ThyraMIR® microRNA/ThyGenX®;
  3. Afirma BRAF after Afirma Genomic Sequencing Classifier; **OR**
  4. Afirma MTC after Afirma Genomic Sequencing Classifier.

C. Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine needle aspirates of the thyroid not meeting criteria outlined above, including, but not limited to, use of RosettaGX Reveal and single-gene *TERT* testing, are considered **experimental / investigational**.

POLICY GUIDELINES
A. In individuals who do not undergo surgical biopsy or thyroidectomy on the basis of gene expression classifier or molecular marker results, regular active surveillance is indicated.

B. Use of molecular marker testing based on fine needle aspirate of a thyroid nodule to rule in malignancy prior to surgical biopsy may guide surgical planning, particularly factors such as choice of surgical facility provider to ensure that the capability is available to conduct a frozen section pathologic reading during surgical biopsy so that surgical approach may be adjusted accordingly in a single surgery.

C. **Genetic Counseling**
    Experts recommend formal genetic counseling for individuals who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some individuals ; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that

genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

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RATIONALE
This evidence review has been updated regularly with searches of the PubMed database. The most recent literature update was performed through June 13, 2024.

Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Evidence reviews assess the evidence on whether a test is clinically valid and clinically useful. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.

Promotion of greater diversity and inclusion in clinical research of historically marginalized groups (e.g., People of Color [African-American, Asian, Black, Latino and Native American]; LGBTQIA (Lesbian, Gay, Bisexual, Transgender, Queer, Intersex, Asexual); Women; and People with Disabilities [Physical and Invisible]) allows policy populations to be more reflective of and findings more applicable to our diverse members. While we also strive to use inclusive language related to these groups in our policies, use of gender-specific nouns (e.g., women, men, sisters, etc.) will continue when reflective of language used in publications describing study populations.

MOLECULAR TESTS TO RULE OUT MALIGNANCY

Clinical Context and Test Purpose
One purpose of molecular testing in individuals with indeterminate findings on fine needle aspirate(s) (FNA) of thyroid nodules is to rule out malignancy and eliminate the need for surgical biopsy or resection.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with indeterminate findings on FNAs of thyroid nodules who would be willing to undergo watchful waiting, depending on the results of their molecular testing. Individuals with indeterminate findings after FNA of thyroid nodule presently proceed to surgical biopsy or resection.

Interventions
The test being considered is molecular testing, which includes either Afirma GSC (Gene Sequencing Classifier) (predicate Afirma GEC [Gene Expression Classifier]) or RosettaGX Reveal.

Comparators
The following practice is currently being used: standard surgical management through surgical biopsy or resection for biopsy.

Outcomes
The potential beneficial outcome of primary interest would be avoiding an unneeded surgical biopsy or resection (e.g., lobectomy or hemithyroidectomy) in a true-negative thyroid nodule that is benign.

Potential harmful outcomes are those resulting from false-negative test results, which may delay diagnosis and surgical resection of thyroid cancer. For small, slow-growing tumors, it is uncertain that a delay in diagnosis would necessarily worsen health outcomes.

The time frame for evaluating the performance of the test is the time from the initial FNA to surgical biopsy or resection measured in weeks to months following an indeterminate result. Papillary thyroid cancer (PTC) is indolent, and a nodule could be observed for many years to ensure no clinical change. Specifically, the American College of Radiology Thyroid Imaging, Reporting and Data System (TI-RADS) recommends surveillance of suspicious nodules through 5 years.[19,]

Study Selection Criteria
For the evaluation of clinical validity of the molecular testing, studies that meet the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

AFIRMA GSC

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Reviews
Lee et al (2022) performed a systematic review and meta-analysis on the diagnostic performance of molecular tests in the assessment of indeterminate thyroid nodules.[20,] Inclusion criteria for trials included indeterminate thyroid results via FNA that included Bethesda categories III and IV, conclusive histopathological results in a group of benign and suspicious changes, and the use of Afirma GSC, ThyroSeq v3, and ThyGeNext as index tests. Investigators identified 7 studies on

Afirma GSC: 1 prospective study by Livhits et al (2021), described below, and 6 retrospective studies. Pooled data for GSC studies on 472 thyroid nodules demonstrated a sensitivity of 96.6% (95% confidence interval [CI], 89.7% to 98.9%), specificity of 52.9% (95% CI, 23.4% to 80.5%), positive predictive value (PPV) of 63% (95% CI, 51% to 74%), and negative predictive value (NPV) of 96% (95% CI, 94% to 98%). Limitations of this meta-analysis include the scarcity of available cohort analyses of the molecular tests and the lack of long-term findings.

Nasr et al (2023) performed a meta-analysis of 13 real-world postvalidation studies (N=1976 patients with indeterminate thyroid nodules) of the Afirma GSC platform and compared results to the validation study by Patel et al (2018, described below).[21,] Studies performed prior to publication of the validation study and commercial availability of Afirma GSC were excluded. Among 11 studies reporting histopathological results for patients who underwent surgery, sensitivity was 97.2% (95% CI, 1.7% to 99.1%; I^2=0%), specificity was 87.7% (95% CI, 83.2% to 91.0%; I^2=63%), PPV ranged from 49.3% (including patients with suspicious molecular testing results who did not undergo surgery; 95% CI, 41.3% to 57.4%; I^2 not reported) to 64.9% (excluding patients with suspicious molecular testing results who did not undergo surgery; 95% CI, 54.4% to 74.1%; I^2=79%), and NPV was 99.5% (95% CI, 98.0% to 99.9%; I^2=0%). Specificity, PPV (excluding patients with suspicious results who did not undergo surgery), and NPV were significantly improved compared to the values reported in the validation study (p<.05 for each comparison).

Prospective Clinical Validation
Patel et al (2018) reported a validation study for the Afirma GSC test. The study included 210 thyroid nodules from 183 patients that had indeterminate results (Bethesda III or IV) on FNA, see Table 3.[22,] All FNA samples had been previously used in the validation of the Afirma GEC test as reported by Alexander et al (2012) in a 19-month, prospective, multicenter (49 academic and community sites) study.[23,] Patel et al (2018) used the banked samples which were reassayed with next-generation sequencing (NGS) for the Afirma GSC validation study.[22,] The previous central, blinded postoperative consensus histopathological diagnosis was used as the reference standard (210 samples) and all personnel were blinded to the other outcomes. The sensitivity of the Afirma GSC study was 91.1% with a specificity of 68.3% and NPV of 96.1% (see Table 4). There were 4 false negatives in patients with malignant nodules who would have been assigned for active observation. In comparison, Afirma GEC correctly identified 78 of 85 malignant nodules as suspicious (92% sensitivity; 95% CI, 84% to 97%) with specificity of 52% (95% CI, 44% to 59%). The NPV ranged from 85% for "suspicious cytologic findings" to 95% for "atypia of undetermined clinical significance." With sensitivity that was similar to the Afirma GEC test, the Afirma GSC improved specificity. There were no notable study limitations.

Livhits et al (2021) published a randomized, controlled study that compared the Afirma GSC test to the ThyroSeq v3 test in patients with thyroid nodules with indeterminate FNA results (Bethesda III or IV).[24,] The study reported clinical validity for both tests; the results of the Afirma GSC test are summarized in Table 3 and Table 4. The study used histopathologic review by expert thyroid pathologists as the reference standard. The study included 201 nodules in the Afirma GSC group. The sensitivity of Afirma GSC was 100%, specificity was 79.6%, and the NPV was 100%. A limitation of the study is that the pathologists who interpreted the histopathologic diagnosis were not blinded to the results of the molecular test. Patients in this trial who were managed nonoperatively were prospectively surveilled via ultrasound for 12 to 60 months, with

results of surveillance reported with median follow-up of 31.8 months.[25,] Among the nodules initially managed nonoperatively, 44 patients were lost to follow-up without surveillance imaging and were excluded from the analysis, with surveillance data available for 195 nodules. Over the course of surveillance, 84% of nodules with benign or negative molecular testing remained stable. Among the 26 nodules with benign or negative molecular testing that exhibited growth on ultrasound, 12 underwent surgery, with 11 histopathologically diagnosed as benign; the 1 malignant nodule was diagnosed as a minimally invasive Hürthle cell carcinoma. Among 33 nodules with suspicious or positive molecular testing that were initially managed nonoperatively (due to patient preference or other reasons), 15 were ultimately resected, 6 of which were benign. In surgically-confirmed cases, the sensitivity of the Afirma GSC and ThyroSeq v3 tests was 100% and 97%, respectively; specificity was 40% and 38%, PPV was 57% and 64%, and NPV was 100% and 92%, respectively (p>.05 for all comparisons between test platforms).

Table 3. Study Characteristics for Afirma GSC

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors	Comment
Patel et al (2018)[22,]	183 patients with 210 indeterminate thyroid nodules by FNA	Multicenter, non-concurrent prospective validation trial	Consensus histopathology diagnosis		Central, blinded histopathological review from Alexander et al (2012)	Assessors were blinded to the pathology	Samples were previously used to validate Afirma GEC
Livhits et al (2021)[24,]	201 indeterminate thyroid nodules by FNA (Afirma GSC)*	Multicenter, randomized controlled trial	Histopathologic diagnosis	Classified as malignant or benign	Samples were tested after surgery	Assessors were unblinded to results of molecular testing	

FNA: Fine needle aspirate; Afirma GEC: gene expression classifier; Afirma GSC: gene sequencing classifier.
*Study included a comparator group assigned to ThyroSeq (reported below)

Table 4. Clinical Validity for Afirma GSC

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% Confidence Interval)			
					Sensitivity	Specificity	PPV	NPV
Patel et al (2018)[22,]	210 nodules	191 nodules	19 with insufficient residual RNA		91.1 (79 to 98)	68.3 (60 to 76)	47.1 (36 to 58)	96.1 (90 to 99)

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% Confidence Interval)			
Livhits et al (2021)[24,]	201 assigned to Afirma GSC	180 nodules	21 were excluded		100 (88.8 to 100)	79.6 (71.7 to 86.1)	53.5 (39.9 to 66.7)	100 (96.6 to 100)

Afirma GSC: gene sequencing classifier; NPV: negative predictive value; PPV: positive predictive value; RNA: ribonucleic acid.

Retrospective Clinical Validation

Meta-analyses have been performed with studies reporting on the performance of the predicate Afirma GEC in cytologically indeterminate nodules.[26,27,] Retrospective studies are subject to ascertainment bias because a large proportion of individuals with Afirma benign reports did not undergo surgery, which makes determining the sensitivity and specificity of the GEC assay impossible.

Supportive information on the accuracy of benign results can be obtained from studies that report long-term follow-up of individuals with indeterminate FNA cytology and Afirma benign results. There are several studies that reported long-term follow-up of Afirma GEC.[28,29,30,] Valderrabano et al (2019) used the benign call rate and PPV of post-marketing studies for a simulation study, concluding that the initial validation study cohort of Afirma GEC was not representative of the populations in whom the test has been used, raising questions regarding its diagnostic performance.[31,] Because the Afirma GSC used the same validation study, these findings would also apply to Afirma GSC.

Harrell et al (2019) reported a retrospective comparison of Afirma GEC (2011 to July 2017) and Afirma GSC (August 2017 through June 2018) for indeterminate FNA.[32,] Afirma GSC identified fewer indeterminate nodules as suspicious (54/139, 38.8%) compared to GEC (281/481, 58.4%) and led to a lower surgery rate, decreasing from 56% in the GEC group to 31% in the GSC group. A similar retrospective comparison was conducted by Polavarapu et al (2021), comparing Afirma GEC and Afirma GSC for indeterminate FNA between January 2013 through December 2019.[33,] Of the 468 indeterminate thyroid nodules included, no molecular testing was performed in 273, 71 had GEC, and 124 had GSC. Use of Afirma GSC led to a lower surgery rate (39.5%; p=.0001) compared to GEC (59.2%) and no molecular testing (67.8%). Additionally, malignancy rate was 20% with no molecular testing, 22% in GEC, and 39% in GSC (p=.022). Afirma GEC benign cell rate was 46%; sensitivity was 100%, specificity was 61%, NPV was 100%, and PPV was 28%. With Afirma GSC, benign cell rate was 60%, sensitivity was 94%, specificity was 76%, NPV was 97%, and PPV was 41%. In conclusion, Afirma GSC testing had a significant reduction in surgical rates and increase in malignancy rates. Sensitivity and NPV were high for both GEC and GSC. A 2023 retrospective analysis of 408 indeterminate thyroid nodules compared the Afirma GSC + XA (n=40), Afirma GEC + GSC (n=255), and Interpace Diagnostics ThyGeNEXT + ThyraMIR platforms (n=113).[34,] Patients either underwent surgery (56.4%) or were monitored for at least 6 months with ultrasound imaging. Sensitivity of the GSC + XA platform was greater than the GEC + GSC platform (80.0% vs 75.81%; p<.001) but not the ThyGeNEXT + ThyraMIR platform (47.4%; p=.08); this may be attributable to the relatively small size of the GSC + XA

group. Specificity of the Afirma GSC + Xa (91.4%) and ThyGeNEXT + ThyraMIR platforms (88.3%) was greater than the GEC + GSC platform (45.1%; p<.001 for both comparisons). NPV was >85% for all cohorts and was highest with the GSC + XA platform (97.0%).

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

No evidence directly demonstrating improved outcomes in patients managed with the Afirma GEC was identified.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Because no direct evidence of utility was identified, a chain of evidence was developed, which addresses 2 key questions:
1. Does use of the Afirma GEC in individuals with cytologically indeterminate thyroid nodules change clinical management (in this case, reduced thyroid resections)?
2. Do those management changes improve outcomes?

Changes in Management

The clinical setting in which the Afirma GEC is meant to be used is well-defined: individuals with atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) or follicular neoplasm or who are suspicious for follicular neoplasm (SFN) on FNA, who do not have other indications for thyroid resection (i.e., in whom the GEC results would play a role in surgical decision making). Decision impact studies, most often reporting on clinical management changes but not on outcomes after surgical decisions were made, have suggested that, in at least some cases, surgical decision making changed.[35,36,37,] [38,39,] It cannot be determined from these studies whether the changes in management improved health outcomes.

Improved Outcomes

A simplified decision model was developed for use with Afirma GEC (which can also be applied to use of the Afirma GSC) in individuals with cytologically indeterminate FNA samples. It is assumed that when Afirma GEC/GSC is not used, patients with cytologically indeterminate FNA results undergo thyroid resection. When Afirma GEC/GSC is used, those with Afirma suspicious lesions undergo resection, while those who have Afirma benign lesions do not. In this case, compared with the standard care plan, some patients without cancer will have avoided a biopsy, which is weighed against the small increase in missed cancers, in patients who had cancer but tested as Afirma benign.

Assuming that the rate of cancer in cytologically indeterminate thyroid nodules is approximately 20%,[40,] in the standard care plan, 80% of patients with cytologically indeterminate FNA samples will undergo an unnecessary biopsy. Applying the test characteristic values from Alexander et al (2012),[23,] it is estimated that approximately 1.6% of individuals with true cancer would be missed, but approximately 38%, instead of 80%, would undergo unneeded surgery. The study by Kim et al (2023), described previously above, reported only 1 false-negative case among 15 patients with nodules demonstrating growth on surveillance imaging over 3 years who underwent delayed surgery, suggesting that the rate of false-negative results and avoided unnecessary surgeries may be further improved with the Afirma GSC and ThyroSeq v3 platforms.[25,]

Whether the tradeoff between avoiding unneeded surgeries and the potential for missed cancer is worthwhile depends, in part, on patient and physician preferences. However, some general statements may be made by considering the consequences of a missed malignancy and the consequences of unnecessary surgery. Most missed malignancies will be PTCs, which have an indolent course. Thyroid nodules are amenable to ongoing surveillance (clinical, ultrasound, and with repeat FNAs), with minimal morbidity.

Thyroid resection is a relatively low-risk surgery. However, the consequences of surgery can be profound. Patients who undergo a hemi- or subtotal thyroidectomy have a risk of recurrent laryngeal nerve damage and parathyroid gland loss. The standard of care for thyroid nodules is based on an intervention that is stratified by FNA cytology results, which are grouped into categories with differing prognosis. Avoiding invasive surgery in situations where patients are at very low likelihood of having an invasive tumor is likely beneficial. Among the low-risk population, the alternative to surgical biopsy is ongoing active surveillance.

While the Kim et al (2023) study is encouraging, evidence of improved outcomes through 5 years of surveillance is needed as recommended by the American College of Radiology.[19,]

ROSETTAGX REVEAL

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Lithwick-Yanai et al (2017) described the development and initial clinical validation of the RosettaGX Reveal quantitative real-time polymerase chain reaction assay for 24 microRNA samples in a multicenter, retrospective cohort study using 201 FNA smears.[41,] The results of the clinical validation study are reported in Table 5.

Table 5. Clinical Validity for RosettaGX Reveal

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% Confidence Interval)			
					Sensitivity	Specificity	PPV	NPV
Lithwick-Yanai et al (2017) [41,]	201 FNA smears	189 passing QC	12		85 (74 to 93)	72 (63 to 79)	NR	91 (84 to 96)
		150 with consensus agreement			98 (87 to 100)	78 (69 to 85)	NR	99 (94 to 10[0)]

FNA: fine needle aspirate; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; QC: quality control.

Walts et al (2018) reported a blinded evaluation of RosettaGX Reveal in 81 archived FNA smears that had Afirma GEC results and histopathology.[42,] Afirma GEC had been requested following indeterminate FNA and had classified 74 nodules as suspicious and 7 as benign. The 81 patients underwent surgery based on Afirma GEC results or clinical factors. The final diagnosis from histopathology was 63 benign and 18 malignant thyroid nodules. Reveal classified 14 of the 18 malignant nodules as suspicious for a sensitivity of 77.8% and specificity of 60.3%.

No prospective clinical studies for RosettaGX Reveal were identified.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. No evidence directly demonstrating improved outcomes in patients managed with the RosettaGX Reveal was identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Section Summary: Molecular Tests to Rule Out Malignancy
A systematic review of 1 prospective and 6 retrospective trials demonstrated a high NPV (96%; 95% CI, 94% to 98%), with a recent meta-analysis of real-world postvalidation data indicating significantly better diagnostic performance of the Afirma GSC platform than in its validation study. In a multicenter validation study, Afirma GSC was also reported to have a high NPV (96%; 95% CI, 90% to 99%). These results are consistent with an earlier study on the Afirma GEC in

the same study population and with a randomized controlled trial of Afirma GSC in a similar study population. In other multicenter and single-center studies, there is suggestive evidence that rates of malignancy are low in Afirma patients who are classified as benign. One prospective study with long-term imaging surveillance of 195 nodules initially managed nonoperatively based on negative/benign Afirma GSC or ThyroSeq v3 testing only indicated 1 false-negative case over 31.8 months of follow-up. The available evidence suggests that physician decision making about surgery is altered by Afirma GSC or ThyroSeq v3 results. A chain of evidence can be constructed to establish the potential for clinical utility with Afirma GSC and ThyroSeq v3 testing in cytologically indeterminate lesions, but evidence of improved outcomes must be demonstrated through at least 5 years of surveillance as recommended by the American College of Radiology..

For the RosettaGX Reveal test, 2 retrospective clinical validation studies have been reported. No prospective studies for patients managed with the RosettaGX Reveal were identified, so the clinical validity remains uncertain.

MOLECULAR TESTS TO RULE IN MALIGNANCY

Clinical Context and Test Purpose
The purpose of testing for molecular markers (e.g., single nucleotide variants and gene rearrangements) in individuals with indeterminate findings on FNA of thyroid nodules is to rule in malignancy and to guide surgical approach or management.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with indeterminate findings on FNA(s) of thyroid nodules. Individuals with indeterminate findings would presently proceed to surgical biopsy perhaps with intraoperative pathology consultation (i.e., intraoperative frozen section) if available.

Interventions
The test being considered is testing for molecular markers (e.g., single nucleotide variants and gene rearrangements) with Afirma BRAF and Afirma MTC (medullary thyroid carcinoma) to guide surgical planning to ensure the capability for intraoperative pathologic confirmation of malignancy to adjust to definitive surgery for initial resection if appropriate.

Comparators
The following practices are currently being used: standard surgical management through surgical resection, including a 2-stage surgical biopsy (i.e., lobectomy) followed by definitive surgery (i.e., hemithyroidectomy or thyroidectomy).

Outcomes
The potential beneficial outcome of primary interest is appropriate surgical planning in the preoperative period (e.g., hemithyroidectomy or thyroidectomy when malignancy is predicted). This has the potential benefit of reducing the likelihood of having the individual repeating surgery if a diagnosis is not made on frozen pathology section during the initial surgery if lobectomy is done as a first procedure.

Potential harmful outcomes are those resulting from false-positive results. However, the use of intraoperative confirmation of malignancy through frozen pathology section in individuals with positive molecular marker testing would mitigate any risk of inappropriately performing more extensive thyroidectomy in the absence of malignancy.

The time frame for evaluating the performance of the test varies from the initial FNA to surgical resection to weeks to months following an indeterminate result.

Study Selection Criteria
For the evaluation of clinical validity of the molecular testing, studies that meet the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

GENE EXPRESSION CLASSIFIERS TO PREDICT MALIGNANCY

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence
Less evidence exists on the validity of gene expression profiling to rule in malignancy (specifically, the Afirma BRAF and Afirma MTC tests). Genetic variants can be used to improve the sensitivity and specificity for diagnosing indeterminate FNA of the thyroid, with the goal of identifying variants that predict malignancy in FNA samples.

Fnais et al (2015) conducted a systematic review and meta-analysis of studies reporting on the test accuracy of *BRAF* variant testing in the diagnosis of PTC.[43,] Reviewers included 47 studies with 9924 FNA samples. For all cytologically indeterminate nodules, the pooled sensitivity estimate for *BRAF* variant testing was 31% (95% CI, 6% to 56%). Among nodules suspicious for malignancy on FNA, the pooled sensitivity estimate for *BRAF* variant testing was 52% (95% CI, 39% to 64%; I^2=77%).

Afirma BRAF and Afirma MTC
Diggans et al (2015), described the development and validation of the Afirma BRAF test, for a subset of 213 thyroid nodule FNA samples for which histopathology was available, Afirma BRAF test results were compared with pathologic findings.[18,] Afirma BRAF classified all histopathologically benign samples as *BRAF* V600E-negative (specificity, 100%; 95% CI, 97.4% to 100%). Of the 73 histopathologically malignant samples, the Afirma BRAF test identified 32 as *BRAF*-positive (sensitivity, 43.8%; 95% CI, 32.2% to 55.9%).

In a study describing the development and validation of the Afirma MTC classifier, Kloos et al (2016) evaluated the MTC classifier in a sample of 10,488 thyroid nodule FNA samples referred

for GEC testing.[44,] In this sample, 43 cases were Afirma MTC-positive, of which 42 were considered to be clinically consistent with MTC on pathology or biochemical testing, for a PPV of 97.7% (95% CI, 86.2% to 99.9%).

Genetic Variants Association With Tumor Behavior

The presence of *BRAF* or telomerase reverse transcriptase (*TERT*) variants is strongly associated with malignancy in thyroid nodule FNA samples. *BRAF* or *TERT* variants have also been associated with more aggressive clinicopathologic features in individuals diagnosed with PTC.

Adeniran et al (2011) assessed 157 cases with equivocal thyroid FNA readings (indeterminate and suspicious for PTC) or with a positive diagnosis for PTC and concomitant *BRAF* variant analysis.[1,] The results of histopathologic follow-up correlated with the cytologic interpretations and *BRAF* status. Based on the follow-up diagnosis after surgical resection, the sensitivity for diagnosing PTC was 63.3% with cytology alone and 80.0% with the combination of cytology and *BRAF* testing. No false-positives were noted with either cytology or *BRAF* variant analysis. All PTCs with an extrathyroidal extension or aggressive histologic features were positive for a *BRAF* variant. The authors concluded that patients with an equivocal cytologic diagnosis and a *BRAF* V600E variant could be candidates for total thyroidectomy and central lymph node dissection.

Xing et al (2009) investigated the utility of *BRAF* variant testing of thyroid FNA specimens for preoperative risk stratification of PTC in 190 patients.[45,] A *BRAF* variant in preoperative FNA specimens was associated with poorer clinicopathologic outcomes for PTC. Compared with the wild-type allele, a *BRAF* variant strongly predicted extrathyroidal extension (23% vs 11%; p=.039), thyroid capsular invasion (29% vs 16%; p=.045), and lymph node metastasis (38% vs 18%; p=.002). During a median follow-up of 3 years (range, 0.6 to 10 years), PTC persistence or recurrence was seen in 36% of *BRAF* variant-positive patients and 12% of *BRAF* variant-negative patients, with an odds ratio (OR) of 4.16 (95% CI, 1.70 to 10.17; p=.002). The PPV and NPV for preoperative FNA-detected *BRAF* variant to predict PTC persistence or recurrence were 36% and 88%, respectively, for all histologic subtypes of PTC. The authors concluded that preoperative *BRAF* variant testing of FNA specimens might provide a novel tool to preoperatively identify PTC patients at higher risk for extensive disease (extrathyroidal extension and lymph node metastases) and those more likely to manifest disease persistence or recurrence.

Yin et al (2016) reported on a systematic review and meta-analysis evaluating *TERT* promoter variants and aggressive clinical behaviors in PTC.[46,] Eight eligible studies (N =2035 patients; range, 30 to 507) were included. Compared with wild-type, *TERT* promoter variant status was associated with lymph node metastasis (OR, 1.8; 95% CI, 1.3 to 2.5; p=.001), extrathyroidal extension (OR, 2.6; 95% CI, 1.1 to 5.9; p=.03), distant metastasis (OR, 6.1; 95% CI, 3.6 to 10.3; p<.001), advanced TNM stages III or IV (OR, 3.2; 95% CI, 2.3 to 4.5; p<.001), poor clinical outcome (persistence or recurrence; OR, 5.7; 95% CI, 3.6 to 9.3; p<.001), and mortality (OR, 8.3; 95% CI, 3.8 to 18.2; p<.001).

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Testing for specific variants associated with thyroid cancer (e.g., *BRAF* V600E, *TERT,* and *RET* variants, *RET/PTC* and *PAX8/PPARγ* rearrangements) is generally designed to "rule in" cancer in nodules with indeterminate cytology on FNA.[47,] (Of note, some gene panels, such as the ThyroSeq panel, may have a high enough NPV that their clinical use could also be considered as a molecular marker to predict benignancy; see next section.) A potential area for clinical utility for this type of variant testing would be in informing preoperative planning for thyroid surgery following initial thyroid FNA, such as planning for a hemi- versus a total thyroidectomy or performance of central neck dissection.

In a retrospective analysis, Yip et al (2014) reported on outcomes after implementation of an algorithm incorporating molecular testing of thyroid FNA samples to guide the extent of initial thyroid resection.[48,] The study included a cohort of patients treated at a single academic center at which molecular testing (*BRAF* V600E, *BRAF* K601E, *NRAS* codon61, *HRAS* codon 61, and *KRAS* codon 12 and 13 single nucleotide variants; *RET/PTC1*, *RET/PTC3*, and *PAX8/PPARγ* rearrangements) was prospectively obtained for all FNAs with indeterminate cytology (FLUS, follicular neoplasm, suspicious for malignancy), and for selective FNAs at the request of the managing physician for selected nodules with benign or nondiagnostic cytology. The study also included a second cohort of patients who did not have molecular testing results available. For patients treated with a molecular diagnosis, a positive molecular diagnostic test was considered an indication for an initial total thyroidectomy. Patients with FLUS and negative molecular diagnostic results were followed with repeat FNA, followed by lobectomy or total thyroidectomy if indeterminate pathology persisted. Patients with a follicular neoplasm or suspicious for malignancy results on cytology and a negative molecular diagnostic result were managed with lobectomy or total thyroidectomy.

The sample included 671 patients, 322 managed with and 349 without molecular diagnostics. Positive molecular testing results were obtained in 56 (17% of those managed with molecular diagnostics) patients, most commonly *RAS* variants (42/56 [75%]), followed by *BRAF* V600E (10/56 [18%]) and *BRAF* K601E (2/56 [4%]) variants, and *PAX8/PPARγ* rearrangements (2/56 [4%]). Compared with those managed without molecular diagnostics (63%), patients managed with molecular diagnostics (69%) were nonsignificantly less likely to undergo total thyroidectomy as an initial procedure (p=.08). However, they had nonsignificantly higher rates of central compartment lymph node dissection (21% vs. 15%, p=.06). Across both cohorts, 25% (170/671) of patients had clinically significant thyroid cancer, with no difference in thyroid cancer rates based on the type of initial surgery (26% for total thyroidectomy vs. 22% for lobectomy, p=.3). The incidence of clinically significant thyroid cancer after initial lobectomy (i.e., requiring a 2-stage surgery) was significantly lower for patients managed with molecular diagnostics (17% vs. 43%, p<.001). An indeterminate FNA result had a sensitivity and specificity for the diagnostic of thyroid cancer of 89% and 27%, respectively, with a PPV of 29% and an NPV of 88%. The addition of molecular diagnostics to FNA results increased the specificity for a cancer diagnosis to 95% and the PPV to 82%.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A task force from the American Thyroid Association (2015) published a review with recommendations for the surgical management of FNA-indeterminate nodules using various molecular genetic tests.[49,] This review reported on the estimated likelihood of malignancy in an FNA-indeterminate nodule depending on results of the Afirma GEC test (described above) and other panels designed to rule in malignancy. Depending on the estimated prebiopsy likelihood of malignancy, recommendations for surgery included observation, active surveillance, repeat FNA, diagnostic lobectomy, or oncologic thyroidectomy.

Section Summary: Molecular Tests to Predict Malignancy
The available evidence has suggested that the use of variant testing in thyroid FNA samples is generally associated with high specificity and PPV for clinically significant thyroid cancer. The most direct evidence related to the clinical utility of variant testing for genes associated with malignancy in thyroid cancer comes from a single-center retrospective study that reported surgical decisions and pathology findings in patients managed with and without molecular diagnostics. There is a potential clinical utility for identifying malignancy with higher certainty on FNA if such testing permits better preoperative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. A statement from the American Thyroid Association provides some guidelines for surgeons managing patients with indeterminate nodules. However, adoption of these guidelines in practice and outcomes associated with them is uncertain.

MOLECULAR TESTS TO RULE OUT AND RULE IN MALIGNANCY

Clinical Context and Test Purpose
The purpose of the ThyroSeq v3 test and the combined ThyGeNEXT Thyroid Oncogene Panel plus ThyraMIR microRNA classifier in individuals with indeterminate findings on FNA(s) of thyroid nodules is to predict malignancy and inform surgical planning decisions with positive results using ThyroSeq v3 or the ThyGeNEXT, and if negative, to predict benignancy using ThyraMIR microRNA classifier to eliminate or necessitate the need for surgical biopsy and guide surgical planning.

The following PICO was used to select literature to inform this review.

Populations
The relevant population of interest is individuals with indeterminate findings on FNA(s) of thyroid nodules. Individuals with indeterminate findings presently proceed to surgical resection.

Interventions
The tests being considered are either: (a) the ThyroSeq v3 test or (b) the combined ThyGeNEXT Thyroid Oncogene Panel and ThyraMIR microRNA classifier testing.

Comparators

The following practices are currently being used: surgical biopsy and/or standard surgical management through surgical resection.

Outcomes
The potential beneficial outcomes of primary interest are using a true-negative result to avoid an unneeded surgical biopsy or using a true-positive result to guide surgical resection (e.g., hemithyroidectomy or thyroidectomy).

Potential harmful outcomes are those resulting from false-positive or false-negative test results. False-positive test results can lead to unnecessary surgical biopsy or resection and procedure-related complications. False-negative test results can lead to lack of surgical biopsy or resection for thyroid cancer and delay in diagnosis.

The time frame for evaluating the performance of the test varies from the initial FNA to surgical resection to weeks to months following an indeterminate result.

Study Selection Criteria
For the evaluation of clinical validity of the molecular testing, studies that meet the following eligibility criteria were considered:
- Reported on the accuracy of the marketed version of the technology (including any algorithms used to calculate scores)
- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described.

THYROSEQ V3 TEST

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

REVIEW OF EVIDENCE

Systematic Review
Lee et al (2022) performed a systematic review and meta-analysis on the diagnostic performance of molecular tests in the assessment of indeterminate thyroid nodules (described above).[20,] Inclusion criteria for trials included indeterminate thyroid results via FNA that included Bethesda categories III and IV, conclusive histopathological results in a group of benign and suspicious changes, and the use of Afirma GSC, ThyroSeq v3, and ThyGeNext as index tests. Investigators identified 6 studies on Thyroseq v3: 3 prospective, including Livhits et al (2021) and Steward et al (2019), described below, and 3 retrospective. Only 2 studies on ThyGeNext were identified and were excluded from meta-analysis due to the small sample size. Pooled data for ThyroSeq studies on 560 thyroid nodules demonstrated a sensitivity of 95.1% (95% CI, 91.1% to 97.4%), specificity of 49.6% (95% CI, 29.3% to 70.1%), PPV of 70% (95% CI, 55% to 83%), and NPV of 92% (95% CI, 86% to 97%). Limitations of this meta-analysis include the scarcity of available cohort analyses of the molecular tests and the lack of long-term findings.

Prospective Clinical Validation

Nikiforova et al (2018) reported on the performance of ThyroSeq v3 with 112 genes.[50,] The training sample included 238 surgically removed tissue samples consisting of 205 thyroid tissue samples representing all main types of benign and malignant tumors and nontumoral conditions. The validation sample included an independent set of 175 FNA samples of indeterminate cytology (see Table 6). Using the cutoff identified in the training set, the ThyroSeq v3 sensitivity was 98% (95% CI, 93% to 99%), specificity was 82% (95% CI, 72% to 89%), with accuracy of 91% (95% CI, 86% to 94%) (see Table 7).

Steward et al (2019) conducted a multicenter validation study of ThyroSeq v3 in 256 patients with an indeterminate FNA who had surgery with histopathology (see Table 6).[51,] Histopathology was reviewed by a central pathology panel and both cytologists and pathologists were blinded to the molecular results. For a benign result, ThyroSeq v3 had a sensitivity of 93%, a specificity of 81%, PPV of 68%, and NPV of 97% (see Table 7). Out of 152 test-negative samples, 5 (3%) were false-negatives. There were 105 cases with positive results, defined as cancer or noninvasive follicular thyroid neoplasm with papillary-like features. Two nodules had high-risk *TERT* or *TP53* variants (both positive for cancer), 13 had variants in *BRAF* V600E or *NTRK3*, or *BRAF*, or RET fusions (all positive for cancer), and 60 nodules were positive for variants in *RAS, BRAF K601E, PTEN, IDH2,* or *DICER1* or *PPARF-THADA* fusion (37 [62%] positive for cancer). No major limitations in study design and conduct of this validation study were identified. Because the nodules with low cancer probability genetic alterations were removed for histological analysis, the long-term clinical impact of the genetic alterations could not be determined.

Livhits et al (2021) published a randomized controlled study that compared the ThyroSeq v3 test to the Afirma GSC test in patients with thyroid nodules with indeterminate results (Bethesda III or IV) (as described above).[24,] The study reported clinical validity for both tests; the results of the ThyroSeq v3 test are summarized in Tables 6 and 7. The study included 171 nodules in the ThyroSeq v3 group. The sensitivity of ThyroSeq v3 was 96.9%, specificity was 84.8%, and the NPV was 99%. Long-term surveillance follow-up of nonoperatively-managed nodules in this trial, described in the section above, continued to support high NPV.[25,] A limitation of the study is that pathologists that interpreted the histopathologic diagnosis were unblinded to the molecular test results. Additionally, the median length of surveillance did not reach 5 years as recommended by the American College of Radiology.

Table 6. Study Characteristics of Clinical Validity ThyroSeq v3

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Nikiforov et al (2018)[50,]	175 samples with indeterminate cytology and known surgical follow-up	Retrospective	Histopathologic diagnosis	Cutoffs determined in the training sample	Samples were tested after surgical outcome was known	Unclear

Study	Study Population	Design	Reference Standard	Threshold for Positive Index Test	Timing of Reference and Index Tests	Blinding of Assessors
Steward et al (2019) [51,]	256 patients (286 nodules) with an indeterminate FNA (Bethesda III, IV, or V) and underwent thyroid surgery	Multicenter (10 sites) prospective validation study	Central pathology review	Classified as malignant or NIFPT or benign	Cross-sectional	Yes
Livhits et al (2021)[24,]	171 nodules with indeterminate FNA (Bethesda III, IV) assigned to ThyroSeq v3*	Multicenter, randomized controlled trial	Histopathologic diagnosis	Classified as malignant or benign	Samples were tested after surgery	Assessors were unblinded to results of molecular testing

FNA: fine needle aspirate; Afirma GSC: Gene Sequencing Classifier; NIFPT: noninvasive follicular thyroid neoplasm with papillary-like features.
*Study included a comparator group assigned to Afirma GSC (reported previously)

Table 7. Clinical Validity of ThyroSeq v3

Study	Initial N	Final N	Excluded Samples	Prevalence of Condition	Clinical Validity (95% Confidence Interval)			
					Sensitivity	Specificity	PPV	NPV
Nikiforov et al (2018)[50,]		175			98 (93 to 100)	81 (72 to 89)		
Steward et al (2019) [51,]	286	57	29 (10%)	30%	93 (86 to 97)	81 (75 to 86)	68 (58 to 76)	97 (93 to 99)
Livhits et al (2021)[24,]	171	163	8		96.9 (83.8 to 100)	84.8 (77 to 90.7)	63.3 (48.3 to 76.6)	99 (94.6 to 100)

NPV: negative predictive value; PPV: positive predictive value.

Additional studies describing the clinical validity of the ThyroSeq v2 panel in external settings (outside of the institution where it was developed) have reported on the diagnostic performance to predict malignancy in thyroid nodules that are indeterminate on FNA have been reported (see Table 8). These studies differed from the previous studies in that noninvasive follicular thyroid neoplasm with papillary-like nuclear features was classified as not malignant for calculation of performance characteristics.

Table 8. Additional Clinical Validity Studies of ThyroSeq to Predict Malignancy in Indeterminate Thyroid FNA Samples

Study	Population	Genes and Rearrangements Tested	Insufficient or Inadequate for Analysis	Measures of Agreement (95% CI), %			
				Sen	Spec	PPV	NPV
Valderrabano et al (2017)[52,]	190 indeterminate thyroid nodules	ThyroSeq v2 (60+ genes)	2	70 (46 to 88)	77 (66 to 85)	42 (25 to 61)	91 (82 to 97)
Taye et al (2018)[53,]	156 indeterminate thyroid nodules	ThyroSeq v2 (60+ genes)	3	89 (52 to 100)	43 (29 to 58)	22 (10 to 38)	96 (78 to 99)

CI: confidence interval; FNA: fine needle aspiration; NPV: negative predictive value; PPV: positive predictive value; Sen: sensitivity; Spec: specificity.

Additional studies describing the clinical validity of the genes that comprise the ThyroSeq panel or other individual variants and combinations of variants to predict malignancy in thyroid nodules that are indeterminate on FNA have been reported. The results that pertain to the use of gene testing in indeterminate thyroid nodules are summarized in Table 9.

Table 9. Clinical Validity of Molecular Markers to Predict Malignancy in Indeterminate Thyroid FNA Samples

Study	Population	Genes and Rearrangements Tested	Insufficient or Inadequate for Analysis	Measures of Agreement, %				
				Sen	Spec	PPV	NPV	Acc
Moses et al (2010)[54,]	110 indeterminate thyroid nodules	*BRAF, KRAS, NRAS, RET/PTC1, RET/PTC3, NTRK1*	2	38	95	67	79	77
Ohori et al (2010)[55,]	100 patients with 117 atypia or follicular lesions of uncertain significance	*BRAF, NRAS, HRAS, KRAS, RET/PTC1,RET/PTC3, PAX8/PPARγ*	NR	60	100	100	92	93
Beaudenon-Huibregtse et al (2014)[56,]	53 nodules with	*BRAF, HRAS, KRAS, NRAS,*		48	89	81	64	

Contains Public Information

Study	Population	Genes and Rearrangements Tested	Insufficient or Inadequate for Analysis	Measures of Agreement, %				
				Sen	Spec	PPV	NPV	Acc
	indeterminate or nondiagnostic FNA	*PAX8-PPARγ, RET-PTC1, RET-PTC3*						

Acc: accuracy; FNA: fine needle aspiration; NPV: negative predictive value; NR: not reported; PPV: positive predictive value; PTC: papillary thyroid carcinoma; Sen: sensitivity; Spec: specificity.
[a] FNA-indeterminate nodules.
[b] FNA suspicious nodules.
[c] Atypia of indeterminate significance.
[d] Follicular neoplasm or suspicious for follicular neoplasm.
[e] Suspicious for malignancy.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials. Randomized controlled studies were not identified; however, a retrospective, single-center study found that use of ThyroSeq v3 in a cohort of patients with indeterminate thyroid nodules reduced the surgical resection rate compared to a cohort of patients without molecular testing.[57,] In addition, the risk of malignancy in thyroid nodules with a positive molecular test was higher than those without molecular testing.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

THYGENX THYROID ONCOGENE PANEL AND THYRAMIR MICRORNA CLASSIFIER

Clinically Valid

A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Review of Evidence

Labourier et al (2015) evaluated the diagnostic algorithm combining a 17-variant panel with ThyraMIR on a cross-sectional cohort of thyroid nodules comprised of 109 FNA samples with AUS/FLUS or follicular neoplasm or SFN across 12 endocrinology centers.[58,] A summary of the sensitivity and specificity of the combined test is listed in Table 10.

Table 10. Summary of Clinical Validity for 17-Variant Panel and ThyraMIR on FNA Samples

Groups	No. of Cases	Sensitivity	Specificity	PPV	NPV	Odds Ratio
Cohort (95% CI), %	109	89 (73 to 97)	85 (75 to 92)	74 (58 to 86)	94 (85 to 98)	44 (13 to 151)
AUS/FLUS (95% CI), %	58	94 (73 to 100)	80 (64 to 91)	68 (46 to 85)	97 (84 to 100)	68 (8 to 590)
FN/SFN (95% CI), %	51	82 (57 to 96)	91 (76 to 98)	82 (57 to 96)	91 (76 to 98)	48 (9 to 269)

Adapted from Labourier et al (2015).[58,]
AUS: atypia of undetermined significance; CI: confidence interval; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspiration; NPV: negative predictive value; PPV: positive predictive value; SFN: suspicious for a follicular neoplasm.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence for the clinical utility for the ThyroSeq v2 test and the combined ThyGenX and ThyraMIR diagnostic testing algorithm is lacking.

Chain of Evidence

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

A chain of evidence may be constructed to infer the potential clinical utility of the combined diagnostic testing algorithm. No studies using ThyGenX NGS panel in FNA samples were identified. However, available evidence has suggested that the use of variant testing using NGS in thyroid FNA samples is generally associated with high specificity and PPV for clinically significant thyroid cancer. There is the potential clinical utility for identifying malignancy with higher certainty on FNA if such testing permits better preoperative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. However, the variant analysis does not achieve an NPV sufficiently high enough to identify which patients can undergo active surveillance over thyroid surgery. In the diagnostic algorithm that reflexes to the ThyraMIR after a negative ThyGenX result, patients receiving reflex testing could identify who may undergo active surveillance over thyroid surgery. A single study using a 17-variant panel with ThyraMIR showed an NPV of 94%. Therefore, the high NPV of ThyraMIR has the potential to accurately predict benignancy and triage patients to active surveillance.

Section Summary: Molecular Markers to Rule Out and Rule in Malignancy
Evidence for the clinical validity of the ThyroSeq v3 NGS panel comes from a systematic review of prospective and retrospective studies and a major prospective clinical validity study. In a systematic review including 3 prospective and 3 retrospective clinical validity studies, sensitivity of ThyroSeq v3 was 95.1%, specificity was 49.6%, PPV was 70%, and NPV was 92%. In the prospective clinical validity study, the performance characteristics were sensitivity, 93%; specificity, 81%; PPV, 68%; NPV, 97%. A randomized controlled trial found similar results with ThyroSeq v3. In 2 independent validation studies with a predicate test (ThyroSeq v2) in which noninvasive follicular thyroid neoplasm with papillary-like nuclear features was categorized as not malignant, performance characteristics were lower and variable (sensitivity, 70% to 89%; specificity, 43% to 77%; PPV, 22% to 42%; NPV, 91% to 96%).

Evidence for the clinical validity of combined testing for miRNA gene expression using ThyraMIR and a targeted 17-variant panel comes from 2 retrospective studies using archived surgical specimens and FNA samples. One study combined a 17-variant panel with ThyraMIR testing on archived surgical specimens and resulted in a sensitivity of 85% and specificity of 95%. The second study combined a 17-variant panel (miR*Inform*) with ThyraMIR testing on FNA samples and resulted in a sensitivity of 89%, a specificity of 85%, PPV of 74%, and NPV of 94%. No studies were identified that demonstrated the clinical validity of a combined ThyGenX and ThyraMIR test on FNA samples.

Direct evidence for the clinical utility for the ThyroSeq v2 test and the combined ThyGenX and ThyraMIR reflex testing is lacking. However, available evidence has suggested that testing for gene variants and rearrangements can predict malignancy and inform surgical planning decisions when the test is positive. Pooled retrospective and prospective clinical validation studies of ThyroSeq v2 have reported a combined NPV of 96% (95% CI, 92% to 95%) and PPV of 83% (95% CI, 72% to 95%) and might potentially assist in selecting patient to avoid surgical biopsy if negative and guide surgical planning if positive. The NPV of the ThyGenX to identify patients who should undergo active surveillance over thyroid surgery is unknown. In a reflex testing setting, the high NPV for a microRNA gene expression test used on the subset of patients with a negative result from a variant and gene rearrangement testing may provide incremental information in identifying patients appropriately for active surveillance, but improvements in health outcomes are still uncertain.

Supplemental Information
The purpose of the following information is to provide reference material. Inclusion does not imply endorsement or alignment with the evidence review conclusions.

Clinical Input From Physician Specialty Societies and Academic Medical Centers
While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

2017 Input
Clinical input was sought to help determine whether testing for molecular markers in fine needle aspirates of the thyroid for management of individuals with thyroid nodule(s) with an indeterminate finding on the fine needle aspirates (FNAs) would provide a clinically meaningful improvement in net health outcome and whether the use is consistent with generally accepted medical practice. In response to requests, clinical input on 7 tests for molecular markers was received from 9 respondents, including 1 specialty society-level response, 1 physician from an academic center, and 7 physicians from 2 health systems

Clinical input supports that the following uses provide a clinically meaningful improvement in net health outcome and indicates the uses are consistent with generally accepted medical practice:

For individuals who have FNA of thyroid nodules with indeterminate cytologic findings (i.e., Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) who receive the following types of molecular marker testing to rule out malignancy and to avoid surgical biopsy:
   o   Afirma Gene Expression Classifier; or
   o   ThyroSeq v2

For individuals who have FNA of thyroid nodules with indeterminate cytologic findings or Bethesda diagnostic category V (suspicious for malignancy) who receive the following types of molecular marker testing to rule in the presence of malignancy to guide surgical planning for the initial resection rather than a 2 stage surgical biopsy followed by definitive surgery:
   o   ThyroSeq v2;
   o   ThyraMIR microRNA/ThyGenX;
   o   Afirma BRAF after Afirma Gene Expression Classifier; or
   o   Afirma MTC after Afirma Gene Expression Classifier.

Clinical input does not support whether the use of RosettaGX Reveal testing in FNA of thyroid nodules provides a clinically meaningful improvement in the net health outcome or is consistent with generally accepted medical practice.

Practice Guidelines and Position Statements
Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American Association of Clinical Endocrinologists et al
The American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazone Medici Endocrinologi (2016) updated their joint guidelines on molecular testing for cytologically indeterminate thyroid nodules, stating[59]:
   • "Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the negative predictive values (NPVs) and positive predictive values (PPVs) for molecular testing."

- "Consider the detection of *BRAF* and *RET/PTC* and, possibly, *PAX8/PPARG* and *RAS* mutations if such detection is available."
- "*TERT* [Telomerase reverse transcriptase] mutational analysis on FNA, when available, may improve the diagnostic sensitivity of molecular testing on cytologic samples."
- "Because of the insufficient evidence and the limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules."

For the role of molecular testing for deciding the extent of surgery the following recommendations were made:
- "Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery."

American College of Radiology
The American College of Radiology (ACR; 2017) Thyroid Imaging, Reporting, and Data System (TI-RADS) Committee published a white paper with expert consensus recommendations for FNA biopsy thresholds and imaging surveillance.[19,] Regarding timing of follow-up sonograms, the publication states: "We advocate timing on the basis of a nodule's ACR TI-RADS level, with additional sonograms for lesions that are more suspicious. For a TR5 lesion, we recommend scans every year for up to 5 years. For a TR4 lesion, scans should be done at 1, 2, 3, and 5 years. For a TR3 lesion, follow-up imaging may be performed at 1, 3, and 5 years. Imaging can stop at 5 years if there is no change in size, as stability over that time span reliably indicates that a nodule has a benign behavior. There is no published evidence to guide management of nodules that enlarge significantly but remain below the FNA size threshold for their ACR TI-RADS level at 5 years, but continued follow-up is probably warranted. If a nodule's ACR TI-RADS level increases on follow-up, the next sonogram should be done in 1 year, regardless of its initial level."

American Thyroid Association
The American Thyroid Association (ATA; 2016) updated its guidelines on the management of thyroid nodules and differentiated thyroid cancer in adults.[60,] These guidelines made the following statements on molecular diagnostics in thyroid nodules that are atypia of undetermined significance or follicular lesion of undetermined significance on cytology and follicular neoplasm or suspicious for follicular neoplasm on cytology (see Table 11).

Table 11. Molecular Diagnostics in Thyroid Nodules on Cytology

Recommendation	SOR	QOE
AUS or FLUS		
"For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making."	Weak	Moderate
"If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference."	Strong	Low
FN or SFN		
"Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making."	Weak	Moderate

AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspirate; QOE: quality of evidence; SFN: suspicious for follicular neoplasm; SOR: strength of recommendation.

The guidelines also stated: "there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed."

National Comprehensive Cancer Network
National Comprehensive Cancer Network (v2.2024) guidelines on the treatment of thyroid cancer comment on the use of molecular diagnostics in thyroid cancer.[61,] For thyroid nodules evaluated with FNA, molecular diagnostics may be employed when lesions are suspicious for:
- Follicular or oncocytic neoplasms.
- Atypia of undetermined significance or follicular lesions of undetermined significance.

The guidelines state that molecular diagnostics have not performed well historically for oncocytic carcinoma. The guideline also endorses the ATA and ACR recommendations for nodule surveillance, described previously above.

U.S. Preventive Services Task Force Recommendations
Not applicable.

Ongoing and Unpublished Clinical Trials
Some currently ongoing and unpublished trials that might influence this review are listed in Table 12.

Table 12. Summary of Key Trials

NCT No.	Trial Name	Planned Enrollment	Completion Date
Ongoing			
NCT05025046[a]	Prospective, Blinded, Multi-center Clinical Study of NGS-based Thyroscan Genomic Classifier in the Diagnosis of Thyroid Nodules	400	Jun 2022 (unknown)
NCT02681328	Randomized Trial Comparing Performance of Molecular Markers for Indeterminate Thyroid Nodules	328	Dec 2025
Unpublished			
NCT03170804	Registry for Genomic Profiling of Nodular Thyroid Disease and Thyroid Cancer	200	Jan 2020 (unknown)
NCT02947035	Molecular Testing to Direct Extent of Initial Thyroid Surgery	100	Jun 2023 (completed)

NCT: national clinical trial.
[a] Denotes industry-sponsored or cosponsored trial.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. This may not be a comprehensive list of procedure codes applicable to this policy.

Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

The code(s) listed below are medically necessary ONLY if the procedure is performed according to the "Policy" section of this document.

CPT/HCPCS	
81345	TERT (telomerase reverse transcriptase) (e.g., thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (e.g., promoter region)
81445	Solid organ neoplasm, genomic sequence analysis panel 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis
81479	Unlisted molecular pathology procedure
81546	Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (e.g., benign or suspicious)
0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy")
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
0287U	Oncology (thyroid), DNA and mRNA, nextgeneration sequencing analysis of 112 genes, fine needle aspirate or formalin fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high)

REVISIONS	
03-17-2017	Policy added to the bcbsks.com web site on 02-15-2017 with an effective date of 03-17-2017.
10-01-2017	In Coding section: ▪ Added CPT code: 0018U.
04-15-2018	Updated Description section.
	In Policy section:

REVISIONS	
	▪ In Item A, added "either", "or ThyroSeq v2", "nodules with", "cytologic findings (i.e., Bethesda diagnostic category III [atypia", "or Bethesda diagnostic category IV", "suspicion for a follicular neoplasm", and removed "that are cytologically considered to be" to read, "The use of either the Afirma Gene Expression Classifier or ThyroSeq v2 in fine needle aspirates of thyroid nodules with indeterminate cytologic findings (i.e., Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) may be considered medically necessary in patients who have ALL of the following characteristics." ▪ In Item B, removed "Mutation analysis in fine needle aspirates of the thyroid is experimental / investigational" and added "The use of any of the following types of molecular marker testing or gene variant analysis in fine needle aspirates of thyroid nodules with indeterminate findings (Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) or suspicious findings (Bethesda diagnostic category V [suspicious for malignancy]) to rule in malignancy to guide surgical planning for initial resection rather than a 2-stage surgical biopsy followed by definitive surgery may be considered medically necessary: 1. ThyroSeq v2; 2. ThyraMIR microRNA/ThyGenX; 3. Afirma BRAF after Afirma Gene Expression Classifier; or 4. Afirma MTC after Afirma Gene Expression Classifier. ▪ In Item C, added "genetic variant analysis, and molecular marker testing" and "including, but not limited to, use of RosettaGX Reveal" to read, "Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine needle aspirates of the thyroid not meeting criteria outlined above, including, but not limited to, use of RosettaGX Reveal, are considered experimental / investigational." ▪ Updated Policy Guidelines.
	Updated Rationale section.
	In Coding section: ▪ Added CPT codes: 81445, 81479.
	Updated References section.
09-14-2018	Policy published on 08-15-2018 with an effective date of 09-14-2018.
	Updated Description section.
	In Policy section: ▪ In Item C, added "and single-gene *TERT* testing" to read, "Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine needle aspirates of the thyroid not meeting criteria outlined above, including, but not limited to, use of RosettaGX Reveal and single-gene *TERT* testing, are considered experimental / investigational."
	Updated Rationale section.
	In Coding section: ▪ Added CPT code: 0026U.
	Updated References section.
	Updated Appendix section.
01-01-2019	In Coding section: ▪ Added new CPT code: 81345.
08-28-2019	Updated Description section.
	In Policy section: ▪ In Item A, removed "v2" to read, "The use of either Afirma Gene Expression Classifier or ThyroSeq in fine needle aspirates of thyroid nodules with indeterminate

REVISIONS	
	cytologic findings (i.e., Bethesa diagnostic category III [atypia / follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm / suspicion for a follicular neoplasm]) may be considered medically necessary in patients who have ALL of the following characteristics". ▪ In Item B 1, removed "v2" to read, "ThyroSeq".
	Updated Rationale section.
	Updated References section.
	Removed Appendix section.
03-16-2021	Updated Description section.
	Updated Rationale section.
	Updated References section.
	Added Appendix section.
04-01-2021	In Coding section: • Added CPT code 0245U and 81546 • Removed CPT code 81545
12-02-2021	Updated Description Section
	Updated Rationale Section
	Updated References Section
01-01-2022	In Coding section ▪ Added CPT 0287U
03-08-2022	Updated Coding Section ▪ Added code: 0204U
06-15-2022	Updated Policy Section ▪ Section A: Changed "Afirma Gene Expression" to read " Afirma Genomic Sequencing" ▪ Section B 3: Changed "Afirma Gene Expression" to read " Afirma Genomic Sequencing" ▪ Section B4: Changed "Afirma Gene Expression" to read " Afirma Genomic Sequencing"
09-27-2022	Updated Description Section
	Update Rationale Section
	Updated References Sections
	Removed Appendix
01-03-2023	Updated Coding Section ▪ Updated Nomenclature for 81445
10-02-2023	Updated Description Section
	Update Rationale Section
	Updated Coding Section ▪ Removed ICD-10 Codes
	Updated References Section
01-01-2024	Updated Coding Section ▪ Updated nomenclature for 81445 (eff. 01-01-2024)
07-01-2024	Updated Coding Section ▪ Removed Deleted Code 0204U (eff. 07-01-2024)
10-08-2024	Updated Description Section
	Update Rationale Section
	Updated References Section

REFERENCES
1. Adeniran AJ, Theoharis C, Hui P, et al. Reflex BRAF testing in thyroid fine-needle aspiration biopsy with equivocal and positive interpretation: a prospective study. Thyroid. Jul 2011; 21(7): 717-23. PMID 21568726
2. Chudova D, Wilde JI, Wang ET, et al. Molecular classification of thyroid nodules using high-dimensionality genomic data. J Clin Endocrinol Metab. Dec 2010; 95(12): 5296-304. PMID 20826580
3. Nikiforov YE, Seethala RR, Tallini G, et al. Nomenclature Revision for Encapsulated Follicular Variant of Papillary Thyroid Carcinoma: A Paradigm Shift to Reduce Overtreatment of Indolent Tumors. JAMA Oncol. Aug 01 2016; 2(8): 1023-9. PMID 27078145
4. Nikiforov YE. Molecular diagnostics of thyroid tumors. Arch Pathol Lab Med. May 2011; 135(5): 569-77. PMID 21526955
5. Han PA, Kim HS, Cho S, et al. Association of BRAF V600E Mutation and MicroRNA Expression with Central Lymph Node Metastases in Papillary Thyroid Cancer: A Prospective Study from Four Endocrine Surgery Centers. Thyroid. Apr 2016; 26(4): 532-42. PMID 26950846
6. Yip L, Nikiforova MN, Yoo JY, et al. Tumor genotype determines phenotype and disease-related outcomes in thyroid cancer: a study of 1510 patients. Ann Surg. Sep 2015; 262(3): 519-25; discussion 524-5. PMID 26258321
7. Lin JD, Fu SS, Chen JY, et al. Clinical Manifestations and Gene Expression in Patients with Conventional Papillary Thyroid Carcinoma Carrying the BRAF(V600E) Mutation and BRAF Pseudogene. Thyroid. May 2016; 26(5): 691-704. PMID 26914762
8. Alzahrani AS, Alsaadi R, Murugan AK, et al. TERT Promoter Mutations in Thyroid Cancer. Horm Cancer. Jun 2016; 7(3): 165-77. PMID 26902827
9. Landa I, Ganly I, Chan TA, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. Sep 2013; 98(9): E1562-6. PMID 23833040
10. Liu X, Bishop J, Shan Y, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer. Aug 2013; 20(4): 603-10. PMID 23766237
11. Liu T, Wang N, Cao J, et al. The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene. Oct 16 2014; 33(42): 4978-84. PMID 24141777
12. Xing M, Liu R, Liu X, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol. Sep 01 2014; 32(25): 2718-26. PMID 25024077
13. Song YS, Lim JA, Choi H, et al. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. Cancer. May 01 2016; 122(9): 1370-9. PMID 26969876
14. Nikiforova MN, Wald AI, Roy S, et al. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab. Nov 2013; 98(11): E1852-60. PMID 23979959
15. Sonic Healthcare USA. Thyroseq. Thyroid Genomic Classifier. 2024. https://www.thyroseq.com/physicians/. Accessed June 13, 2024.

16. Barros-Filho MC, Marchi FA, Pinto CA, et al. High Diagnostic Accuracy Based on CLDN10, HMGA2, and LAMB3 Transcripts in Papillary Thyroid Carcinoma. J Clin Endocrinol Metab. Jun 2015; 100(6): E890-9. PMID 25867809

17. Zheng B, Liu J, Gu J, et al. A three-gene panel that distinguishes benign from malignant thyroid nodules. Int J Cancer. Apr 01 2015; 136(7): 1646-54. PMID 25175491

18. Diggans J, Kim SY, Hu Z, et al. Machine learning from concept to clinic: reliable detection of BRAF V600E DNA mutations in thyroid nodules using high-dimensional RNA expression data. Pac Symp Biocomput. 2015: 371-82. PMID 25592597

19. Tessler FN, Middleton WD, Grant EG, et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol. May 2017; 14(5): 587-595. PMID 28372962

20. Lee E, Terhaar S, McDaniel L, et al. Diagnostic performance of the second-generation molecular tests in the assessment of indeterminate thyroid nodules: A systematic review and meta-analysis. Am J Otolaryngol. 2022; 43(3): 103394. PMID 35241290

21. Nasr CE, Andrioli M, Endo M, et al. Real-World Performance of the Afirma Genomic Sequencing Classifier (GSC)-A Meta-analysis. J Clin Endocrinol Metab. May 17 2023; 108(6): 1526-1532. PMID 36470585

22. Patel KN, Angell TE, Babiarz J, et al. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. JAMA Surg. Sep 01 2018; 153(9): 817-824. PMID 29799911

23. Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med. Aug 23 2012; 367(8): 705-15. PMID 22731672

24. Livhits MJ, Zhu CY, Kuo EJ, et al. Effectiveness of Molecular Testing Techniques for Diagnosis of Indeterminate Thyroid Nodules: A Randomized Clinical Trial. JAMA Oncol. Jan 01 2021; 7(1): 70-77. PMID 33300952

25. Kim NE, Raghunathan RS, Hughes EG, et al. Bethesda III and IV Thyroid Nodules Managed Nonoperatively After Molecular Testing With Afirma GSC or Thyroseq v3. J Clin Endocrinol Metab. Aug 18 2023; 108(9): e698-e703. PMID 36995878

26. Santhanam P, Khthir R, Gress T, et al. Gene expression classifier for the diagnosis of indeterminate thyroid nodules: a meta-analysis. Med Oncol. Feb 2016; 33(2): 14. PMID 26749587

27. Liu Y, Pan B, Xu L, et al. The Diagnostic Performance of Afirma Gene Expression Classifier for the Indeterminate Thyroid Nodules: A Meta-Analysis. Biomed Res Int. 2019; 2019: 7150527. PMID 31531363

28. Angell TE, Frates MC, Medici M, et al. Afirma Benign Thyroid Nodules Show Similar Growth to Cytologically Benign Nodules During Follow-Up. J Clin Endocrinol Metab. Nov 2015; 100(11): E1477-83. PMID 26353010

29. Sipos JA, Blevins TC, Shea HC, et al. LONG-TERM NONOPERATIVE RATE OF THYROID NODULES WITH BENIGN RESULTS ON THE AFIRMA GENE EXPRESSION CLASSIFIER. Endocr Pract. Jun 2016; 22(6): 666-72. PMID 26789352

30. Deaver KE, Haugen BR, Pozdeyev N, et al. Outcomes of Bethesda categories III and IV thyroid nodules over 5 years and performance of the Afirma gene expression classifier: A single-institution study. Clin Endocrinol (Oxf). Aug 2018; 89(2): 226-232. PMID 29791966

31. Valderrabano P, Hallanger-Johnson JE, Thapa R, et al. Comparison of Postmarketing Findings vs the Initial Clinical Validation Findings of a Thyroid Nodule Gene Expression

Classifier: A Systematic Review and Meta-analysis. JAMA Otolaryngol Head Neck Surg. Sep 01 2019; 145(9): 783-792. PMID 31318389

32. Harrell RM, Eyerly-Webb SA, Golding AC, et al. STATISTICAL COMPARISON OF AFIRMA GSC AND AFIRMA GEC OUTCOMES IN A COMMUNITY ENDOCRINE SURGICAL PRACTICE: EARLY FINDINGS. Endocr Pract. Feb 2019; 25(2): 161-164. PMID 30383497

33. Polavarapu P, Fingeret A, Yuil-Valdes A, et al. Comparison of Afirma GEC and GSC to Nodules Without Molecular Testing in Cytologically Indeterminate Thyroid Nodules. J Endocr Soc. Nov 01 2021; 5(11): bvab148. PMID 34708178

34. Kandil E, Metz TA, Issa PP, et al. Diagnostic Performance of Afirma and Interpace Diagnostics Genetic Testing in Indeterminate Thyroid Nodules: A Single Center Study. Cancers (Basel). Mar 31 2023; 15(7). PMID 37046759

35. Duick DS, Klopper JP, Diggans JC, et al. The impact of benign gene expression classifier test results on the endocrinologist-patient decision to operate on patients with thyroid nodules with indeterminate fine-needle aspiration cytopathology. Thyroid. Oct 2012; 22(10): 996-1001. PMID 22873825

36. Aragon Han P, Olson MT, Fazeli R, et al. The impact of molecular testing on the surgical management of patients with thyroid nodules. Ann Surg Oncol. Jun 2014; 21(6): 1862-9. PMID 24522987

37. Noureldine SI, Olson MT, Agrawal N, et al. Effect of Gene Expression Classifier Molecular Testing on the Surgical Decision-Making Process for Patients With Thyroid Nodules. JAMA Otolaryngol Head Neck Surg. Dec 2015; 141(12): 1082-8. PMID 26606459

38. Abeykoon JP, Mueller L, Dong F, et al. The Effect of Implementing Gene Expression Classifier on Outcomes of Thyroid Nodules with Indeterminate Cytology. Horm Cancer. Aug 2016; 7(4): 272-8. PMID 27102883

39. Chaudhary S, Hou Y, Shen R, et al. Impact of the Afirma Gene Expression Classifier Result on the Surgical Management of Thyroid Nodules with Category III/IV Cytology and Its Correlation with Surgical Outcome. Acta Cytol. 2016; 60(3): 205-10. PMID 27344463

40. Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. Thyroid. Nov 2009; 19(11): 1159-65. PMID 19888858

41. Lithwick-Yanai G, Dromi N, Shtabsky A, et al. Multicentre validation of a microRNA-based assay for diagnosing indeterminate thyroid nodules utilising fine needle aspirate smears. J Clin Pathol. Jun 2017; 70(6): 500-507. PMID 27798083

42. Walts AE, Sacks WL, Wu HH, et al. A retrospective analysis of the performance of the RosettaGX ® Reveal™ thyroid miRNA and the Afirma Gene Expression Classifiers in a cohort of cytologically indeterminate thyroid nodules. Diagn Cytopathol. Nov 2018; 46(11): 901-907. PMID 30353692

43. Fnais N, Soobiah C, Al-Qahtani K, et al. Diagnostic value of fine needle aspiration BRAF(V600E) mutation analysis in papillary thyroid cancer: a systematic review and meta-analysis. Hum Pathol. Oct 2015; 46(10): 1443-54. PMID 26232865

44. Kloos RT, Monroe RJ, Traweek ST, et al. A Genomic Alternative to Identify Medullary Thyroid Cancer Preoperatively in Thyroid Nodules with Indeterminate Cytology. Thyroid. Jun 2016; 26(6): 785-93. PMID 26992356

45. Xing M, Clark D, Guan H, et al. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. J Clin Oncol. Jun 20 2009; 27(18): 2977-82. PMID 19414674

46. Yin DT, Yu K, Lu RQ, et al. Clinicopathological significance of TERT promoter mutation in papillary thyroid carcinomas: a systematic review and meta-analysis. Clin Endocrinol (Oxf). Aug 2016; 85(2): 299-305. PMID 26732020
47. Bernet V, Hupart KH, Parangi S, et al. AACE/ACE disease state commentary: molecular diagnostic testing of thyroid nodules with indeterminate cytopathology. Endocr Pract. Apr 2014; 20(4): 360-3. PMID 24727662
48. Yip L, Wharry LI, Armstrong MJ, et al. A clinical algorithm for fine-needle aspiration molecular testing effectively guides the appropriate extent of initial thyroidectomy. Ann Surg. Jul 2014; 260(1): 163-8. PMID 24901361
49. Ferris RL, Baloch Z, Bernet V, et al. American Thyroid Association Statement on Surgical Application of Molecular Profiling for Thyroid Nodules: Current Impact on Perioperative Decision Making. Thyroid. Jul 2015; 25(7): 760-8. PMID 26058403
50. Nikiforova MN, Mercurio S, Wald AI, et al. Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. Cancer. Apr 15 2018; 124(8): 1682-1690. PMID 29345728
51. Steward DL, Carty SE, Sippel RS, et al. Performance of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology: A Prospective Blinded Multicenter Study. JAMA Oncol. Feb 01 2019; 5(2): 204-212. PMID 30419129
52. Valderrabano P, Khazai L, Leon ME, et al. Evaluation of ThyroSeq v2 performance in thyroid nodules with indeterminate cytology. Endocr Relat Cancer. Mar 2017; 24(3): 127-136. PMID 28104680
53. Taye A, Gurciullo D, Miles BA, et al. Clinical performance of a next-generation sequencing assay (ThyroSeq v2) in the evaluation of indeterminate thyroid nodules. Surgery. Jan 2018; 163(1): 97-103. PMID 29154079
54. Moses W, Weng J, Sansano I, et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. World J Surg. Nov 2010; 34(11): 2589-94. PMID 20703476
55. Ohori NP, Nikiforova MN, Schoedel KE, et al. Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance". Cancer Cytopathol. Feb 25 2010; 118(1): 17-23. PMID 20099311
56. Beaudenon-Huibregtse S, Alexander EK, Guttler RB, et al. Centralized molecular testing for oncogenic gene mutations complements the local cytopathologic diagnosis of thyroid nodules. Thyroid. Oct 2014; 24(10): 1479-87. PMID 24811481
57. Li W, Justice-Clark T, Cohen MB. The utility of ThyroSeq ® in the management of indeterminate thyroid nodules by fine-needle aspiration. Cytopathology. Jul 2021; 32(4): 505-512. PMID 33914382
58. Labourier E, Shifrin A, Busseniers AE, et al. Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology. J Clin Endocrinol Metab. Jul 2015; 100(7): 2743-50. PMID 25965083
59. Gharib H, Papini E, Garber JR, et al. AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS, AMERICAN COLLEGE OF ENDOCRINOLOGY, AND ASSOCIAZIONE MEDICI ENDOCRINOLOGI MEDICAL GUIDELINES FOR CLINICAL PRACTICE FOR THE DIAGNOSIS AND MANAGEMENT OF THYROID NODULES--2016 UPDATE. Endocr Pract. May 2016; 22(5): 622-39. PMID 27167915

60. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. Jan 2016; 26(1): 1-133. PMID 26462967
61. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology: Thyroid Carcinoma. Version 2.2024. Updated March 12, 2024. https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf. Accessed June 13, 2024.

OTHER REFERENCES
1.   Blue Cross and Blue Shield of Kansas Pathology Liaison Committee, May 2018.