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Title: Genetic Testing- Molecular Markers in Fine Needle Aspirates (FNA) of the Thyroid

Description/Background

Fine needle aspiration (FNA) of a thyroid lesion to identify which patients need to undergo surgery has diagnostic limitations and has led to the development of molecular markers in an attempt to improve the accuracy of patient selection.

THYROID NODULES

Thyroid nodules are common, present in 5% to 7% of the U.S. adult population. Most are benign, and most cases of thyroid cancer are curable by surgery 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 (inclusive, indeterminate, atypical, suspicious), 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 according to Bethesda System criteria into the following groups: nondiagnostic; benign; follicular lesion (FLUS) or atypia of undetermined significance (AUS); follicular neoplasm (or suspicious for follicular neoplasm); suspicious for malignancy; and malignant. Lesions with FNA cytology in the FLUS/AUS or follicular neoplasm category are often considered indeterminate.

Management

There is some individualization of management for patients with FNA-indeterminate nodules, but many patients will ultimately require a surgical biopsy, typically thyroid lobectomy, with intraoperative pathology consultation would typically be the next step in diagnosis. Approximately 80% of patients with indeterminate cytology undergo surgical resection; postoperative evaluation reveals 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, as different thyroid malignancies may require different surgical procedures (e.g., unilateral lobectomy versus 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, etc.) 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 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 an FNA in a case of PTC is indeterminate, intraoperative consultation is most often diagnostic, although its efficacy and therefore use will vary between 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 of blood vessels is diagnostic and cannot be determined by cytology, as 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 mutation 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

Various genetic variants have been discovered in thyroid cancer. The 4 gene variants that are the most common and carry the highest impact on tumor diagnosis and prognosis are *BRAF* and *RAS* point mutations and *RET/PTC* and *PAX8/PPAR γ* rearrangements.

Papillary carcinomas carry point mutations of the *BRAF* and *RAS* genes, as well as *RET/PTC* and *TRK* rearrangements, all of which are able to activate the mitogen-activated protein kinase (MAPK) pathway. These mutually exclusive mutations are found in more than 70% of papillary carcinomas. *BRAF* mutations are highly specific for PTC. Follicular carcinomas harbor either *RAS* mutations or *PAX8/PPAR γ* rearrangement. These mutations are also mutually exclusive and 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 cancer and have higher prevalence in less differentiated thyroid carcinomas.⁴ Additional mutations 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 point mutations located in the *RET* gene.

Studies have evaluated the association between various genes and cancer phenotype in individuals with diagnosed thyroid cancer.⁵⁻⁷

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.⁹⁻¹¹ Also, the co-occurrence of *BRAF* or *RAS* variants with *TERT* or *TP53* variants may identify a subset of thyroid cancers with unfavorable outcomes.¹²⁻¹⁴

MOLECULAR DIAGNOSTIC TESTING

Variant Detection and Rearrangement Testing

Single nucleotide variants (SNVs) in specific genes, including *BRAF*, *RAS*, and *RET*, and evaluation for rearrangements associated with thyroid cancers can be accomplished by gene sequencing with Sanger sequencing or pyrosequencing or by real-time polymerase chain reaction (rtPCR). Panels of tests for mutations associated with thyroid cancer are also available. For example, Quest Diagnostics offers a Thyroid Cancer Mutation Panel, which includes *BRAF* and *RAS* mutation analysis and testing for *RET/PTC* and *PAX8/PPAR γ* rearrangements.

The ThyroSeq® v.3 Next Generation Sequencing panel (CBLPath, Ocala, FL) is a NGS sequencing panel of more than 112 genes. According to the ThyroSeq's manufacturer's website, the test is indicated when FNA cytology indicates atypia of uncertain significance or follicular lesion of undetermined significance, follicular neoplasm or suspicious for follicular neoplasm, or suspicious for malignancy.¹⁵ It has been evaluated in patients with follicular neoplasm/suspicious for follicular neoplasm on FNA as a test to increase both sensitivity and specificity for cancer diagnosis.

The ThyGenX™ Thyroid Oncogene Panel (formerly miRInform® Thyroid; Interpace Diagnostics, Parsippany, NJ; testing done at Asuragen Clinical Laboratory) is a NGS sequencing 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 accessed through the use of gene expression profiling, which refers to analysis of messenger RNA (mRNA) expression levels of many genes simultaneously. Several gene expression profiling tests are now available to biologically stratify tissue from thyroid nodules.

The Afirma® Gene Expression Classifier (Afirma GSCGSC; Veracyte, South San Francisco, CA) analyzes the expression of 142 different genes to determine patterns associated with benign findings on surgical biopsy. It is designed to be used for thyroid nodules that have an “indeterminate” classification on FNA as a method to select patients who are at low risk for cancer (“rule out”). In 2017, Veracyte migrated the Afirma GSCGSC 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 1,115 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™ (Interpace Diagnostics, Parsippany, NJ) is a microRNA expression based classifier that is 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 GSC 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* mutations (Afirma BRAF) or mutations associated with medullary thyroid carcinoma (Afirma MTC). Table 1 describes the testing algorithm for Afirma MTC and Afirma BRAF.

Table 1. Afirma MTC and Afirma BRAF Testing Algorithm

Test 1	Test 1 Result	Reflex to Test 2
Thyroid nodule on FNA	“Intermediate”	Afirma MTC
Afirma GSCGSC	“Malignant” or “suspicious”	Afirma MTC
Afirma GSCGSC	“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* mutations: (1) PCR-based methods may have low sensitivity, requiring that a large proportion of the nodule have a relevant mutation; (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 a 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 a central neck dissection.

Algorithmic Testing Using ThyGenX and ThyraMIR

The ThyGenX™ Thyroid Oncogene Panel (Interpace Diagnostics, Parsippany, NJ; testing done at Asuragen Clinical Laboratory) is a NGS panel designed to assess patients with indeterminate thyroid FNA results. It includes sequencing of 8 genes associated with papillary thyroid carcinoma and follicular carcinomas. ThyGenX has replaced the predicate test miRInform® Thyroid that tested for 17 validated gene alterations.

ThyraMIR™ (Interpace Diagnostics, Parsippany, NJ) is a micro-RNA 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 to first 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 NPV 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

Testing for mutations associated with thyroid cancer via sequencing or rtPCR are laboratory-developed tests (LDTs). Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Improvement Act (CLIA). Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing.

In 2013, the U.S. Food and Drug Administration (FDA) approved through the premarket approval process the THxID™-BRAF kit, which is an in vitro diagnostic device to assess specific *BRAF* mutations in melanoma tissue via rtPCR. However, there are currently no diagnostic tests for thyroid cancer mutation analysis with approval from FDA.

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® GSCGSC	Afirma®--GSC	mRNA gene expression	1,115 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 , miRInform® ^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	<ul style="list-style-type: none"> • Benign • Suspicious for malignancy • High risk for medullary carcinoma

FNA: fine needle aspirate; NGS: next-generation sequencing; PCR: polymerase chain reaction.

^a The miRInform® test is the predicate test to ThyGenX™ and is not commercially available.

^b includes TERT

^c Available literature on TERT testing used PCR

Medical Policy Statement

For individuals who have thyroid nodules without strong clinical or radiologic findings suggestive of malignancy in whom surgical decision making would be affected by test results, the use of either of the following types of molecular marker testing or gene variant analysis 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]) is established:

- Afirma® Genomic Sequencing Classifier; or
- ThyroSeq®

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 **established**:

- ThyroSeq;
- ThyraMIR® microRNA/ThyrGeNEXT®;
- Afirma BRAF after Afirma Genomic Sequencing Classifier; or
- Afirma MTC after Afirma Genomic Sequencing Classifier.

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**.

Inclusionary and Exclusionary Guidelines

N/A

CPT/HCPCS Level II Codes *(Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)*

Established codes:

81445	81546	0018U	0026U	0208U	0245U	81479*
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*When used for some Afirma testing

Other codes (investigational, not medically necessary, etc.):

81479** 81345

**When used for RosettaGX Reveal testing

Rationale

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.

MOLECULAR MARKERS TO RULE OUT MALIGNANCY

Clinical Context and Test Purpose

The purpose of molecular testing in individuals with indeterminate findings on fine needle aspirate(s) (FNA) of thyroid nodules to rule out malignancy and eliminate the need for surgical resection.

The relevant question addressed in this evidence review is: Does molecular testing appropriately eliminate the need for surgical resection and lead to improved health outcomes?

The following **PICOs** were used to select literature to inform this review.

Populations

The relevant population of interest includes individuals with indeterminate findings on FNAs of thyroid nodules who would be willing to undergo watchful waiting, depending on results of their molecular testing. Patients with indeterminate findings presently proceed to surgical resection.

Interventions

The relevant intervention of interest is molecular testing which includes Afirma GSC, ThyroSeq v3, or RosettaGX Reveal.

Comparators

The relevant comparator of interest is standard surgical management through surgical resection.

Outcomes

The potential beneficial outcomes of primary interest are avoiding an unneeded surgical resection (e.g., hemithyroidectomy or thyroidectomy) due to thyroid nodules that are absent of cancer.

Potential harmful outcomes are those resulting from false-negative test results, which may delay diagnosis and surgical resection for 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 performance of the test varies the time from the initial fine needle aspiration to surgical resection to weeks to months following an indeterminate result to years. Papillary thyroid cancer is an indolent cancer, 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

Afirma GSC (CPT 81545, 81479)

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.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 fine needle aspirate (FNA, see Table 3).²² All FNA samples had been previously used in the validation of the Afirma GSC test as reported by Alexander et al (2012) in a 19-month, prospective, multicenter (49 academic and community sites) study.²¹ A total of 4812 nodules were screened for inclusion with centralized cytopathology. Local pathology reports of the cytologic diagnosis were collected for all patients, and reports without a definitive benign or malignant diagnosis at the local site were reviewed by 3 expert cytopathologists, who reclassified them as atypical, follicular neoplasm, or suspicious for a follicular neoplasm, or suspicious for malignancy. Of all nodules screened, 577 (12%) were considered indeterminate after central review, and 413 of those had tissue pathology available for a blinded histopathological reference standard. After exclusion of 25 used for test validation and those without a valid GSC result, 265 indeterminate FNA samples were evaluated with the Afirma GSC. Of the 265 samples, 85 nodules were malignant; the GSC correctly identified 78 of the 85 as suspicious (92% sensitivity; 95% CI, 84% to 97%). Specificity was 52% (95% CI, 44% to 59%). The NPV ranged from 85% for "suspicious cytologic findings" to 95% for "atypia of undetermined clinical significance." Seven FNAs had false-negative results, six of which were thought to be due to hypocellular aspirate specimens.

The study reported by Patel et al (2018) used the banked samples which were reassayed with NGS for the Afirma GSC validation study.²⁰ 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. Sensitivity of the Afirma GSC study was 91.1% with specificity of 68.3% and negative predictive value of 96.1% (95% Confidence Interval [CI] 90 to 99) (see Table 4). There were four false negatives in patients with malignant nodules who would have been assigned for active observation. With sensitivity that was similar to the Afirma GSC test, the specificity was improved with Afirma GSC. 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).²² 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%. However, one limitation of the study included that most indeterminate nodules with benign molecular tests were managed nonoperatively; therefore, false negative cases might not have been identified. In addition, 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 ^b
Patel et al (2018)	183 patients with 210 indeterminate thyroid nodules by FNA	Multicenter, nonconcurrent 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 GSC
Livhits et al (2021)	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 GSC: 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)	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)
Livhits et al (2021)	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)

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. Sipos JA, Blevins TC, Shea HC, et al.²⁸⁻³⁰ 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. No studies were identified with long-term follow-up after Afirma GSC tests.

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%; $p=0.0001$) 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 ($p=0.022$). 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%; $p<0.001$) but not the ThyGeNEXT + ThyraMIR platform (47.4%; $p=0.08$); 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%; $p<0.001$ 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 GSC 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 GSC 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 GSC is meant to be used is well-defined: individuals with atypia of undetermined significance (AUS) or follicular lesions 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 GSC 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.³⁵⁻³⁹

Improved Outcomes

A simplified decision model was developed for use with Afirma GSC in individuals with cytologically indeterminate FNA samples. It is shown in Appendix Figure 1. 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, 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.

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 (CPT 81479)

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).

Lithwick-Yanai et al (2017) described the development and initial clinical validation of using the RosettaGX Reveal quantitative rt-PCR assay for 24 microRNAs in a multicenter, retrospective cohort study using 201 FNA smears. The results of the clinical validation study are reported in Table 5.⁴¹

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)	201 FNA smears	189 passing QC	12		85 (74-93)	72 (63-79)	NR	91 (84-96)
		150 with consensus agreement			98 (87-100)	78 (69-85)	NR	99 (94-100)

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 GSC results and histopathology.⁴² Afirma GSC 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 GSC 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.

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 Markers 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 reported to have a high (NPV 96%; 95% CI, 90%-99%). These results are consistent with an earlier study on the Afirma GSC in the same study population and with a RCT 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, but the exact NPV is unknown. 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 or 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, two 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 MARKERS TO RULE IN MALIGNANCY

Clinical Context and Test Purpose

The purpose of testing for molecular markers (e.g., single nucleotide variants [SNVs] and gene rearrangement) in individuals with indeterminate findings on fine needle aspirate(s) of thyroid nodules is to predict malignancy and change surgical approach or management.

The following **PICOs** were used to select literature to inform this review.

Populations

The relevant population of interest includes individuals with indeterminate findings on fine needle aspirate(s) of thyroid nodules. Patients with indeterminate findings presently proceed to

surgical biopsy with intraoperative pathology consultation (i.e., intraoperative frozen section) if available.

Interventions

The relevant intervention of interest is testing for molecular markers (e.g., single nucleotide variants and gene rearrangements to predict malignancy and to use molecular marker results that are positive for variants associated with malignancy to guide surgical planning to ensure the capability for intraoperative pathologic confirmation of malignancy in order to adjust to definitive surgery for initial resection if appropriate.

Comparators

The relevant comparator of interest is 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 outcomes of primary interest are to allow for appropriate surgical planning in the preoperative period (e.g., hemithyroidectomy or thyroidectomy when malignancy is predicted or lobectomy if malignancy is less likely). This has the potential benefit of reducing the likelihood of having the patient have to have repeat 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 or false-negative test results. False-positive test results can lead to unnecessary surgical resection and procedure-related complications. False-negative test results can lead to lack of surgical resection for thyroid cancer.

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

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, and *TERT* single-gene testing). 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

In the study by Diggans et al (2015), describing the development and validation of the Afirma BRAF test (previously described), 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 GSC 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 positive predictive value (PPV) of 97.7% (95% CI, 86.2% to 99.9%).

Genetic Variants Association with Tumor Behavior

The presence of *BRAF* or *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%; $p=0.039$), thyroid capsular invasion (29% vs 16%; $p=0.045$), and lymph node metastasis (38% vs. 18%; $p=0.002$). During a median follow-up of 3 years (range, 0.6-10 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; $p=0.002$). 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 (total $n=2035$

patients; range, 30-507) 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=0.001), extrathyroidal extension (OR=2.6; 95% CI, 1.1 to 5.9; p=0.03), distant metastasis (OR=6.1; 95% CI, 3.6 to 10.3; p<0.001), advanced TNM stages III or IV (OR=3.2; 95% CI, 2.3 to 4.5; p<0.001), poor clinical outcome (persistence or recurrence; OR=5.7; 95% CI, 3.6 to 9.3; p<0.001), and mortality (OR=8.3; 95% CI, 3.8 to 18.2; p<0.001).

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 mutations associated with thyroid cancer (e.g., *BRAF* V600E mutations, *RET* mutations, and *RET/PTC* and *PAX8/PPAR γ* rearrangements) are generally designed to “rule in” cancer in nodules that have indeterminate cytology on FNA.⁴⁷ (Of note, some mutation 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 mutation 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 a central neck dissection.

In a retrospective analysis, Yip et al (2014) reported outcomes after implementation of an algorithm incorporating molecular testing of thyroid FNA samples to guide the extent 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* codon 61, *HRAS* codon 61, and *KRAS* codon 12 and 13 point mutations; *RET/PTC1*, *RET/PTC3*, and *PAX8/PPAR γ* rearrangements) was prospectively obtained for all FNAs with indeterminate cytology (follicular lesion of undetermined significance, follicular neoplasm, and suspicious for malignancy), and for selective FNAs at the request of the managing physician for selected nodules with either benign or nondiagnostic cytology. The study also included a second cohort of patients who did not have molecular testing results available. For the patients treated with molecular diagnosis, a positive molecular diagnostic test was considered to be an indication for an initial total thyroidectomy. Patients with follicular lesion of undetermined significance and negative molecular diagnostic results were followed with repeat FNA, followed by a lobectomy or total thyroidectomy if indeterminate pathology persisted. Patients with 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 and 349 managed with and without molecular diagnostics, respectively. Positive molecular testing results were obtained in 56 patients (17% of those managed with molecular diagnostics), most commonly *RAS* mutations (42/56 [75%]), followed by *BRAF* V600E (10/56 [18%]), *BRAF* K601E (2/56 [4%]), and *PAX8/PPAR γ* rearrangements (2/56 [4%]). Compared with those managed without molecular diagnostics,

patients managed with molecular diagnostics were nonsignificantly less likely to undergo total thyroidectomy as an initial procedure (63% vs. 69%, $p=0.08$). However, they had nonsignificantly higher rates of central compartment lymph node dissection (21% vs. 15%, $p=0.06$). Across both cohorts, 25% of patients (170/671) were found to have clinically significant thyroid cancer, with no difference in thyroid cancer rates based on the type of initial operation (26% for total thyroidectomy vs. 22% for lobectomy, $p=0.3$). 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%, $p<0.001$). An indeterminate FNA result had sensitivity and specificity for the diagnostic of thyroid cancer of 89% and 27%, respectively, with PPV and NPV of 29% and 88%, respectively. 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.

In 2015, a task force from the American Thyroid Association (ATA) reported on a review with recommendations for the surgical management of FNA-indeterminate nodules with 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 GSC (described above) and other panels designed to rule in malignancy. Depending on the estimated prebiopsy likelihood of malignancy, recommendations for surgery include observation, active surveillance, repeat FNA, diagnostic lobectomy, or oncologic thyroidectomy.

Section Summary: Molecular Markers to Predict Malignancy

The available evidence suggests that the use of mutation testing in thyroid FNA samples is generally associated with a high specificity and PPV for clinically significant thyroid cancer. The most direct evidence related to the clinical utility of mutation testing for genes associated with malignancy in thyroid cancer comes from one single-center retrospective study, which reported surgical decisions and pathology findings in patients managed with and without molecular diagnostics. There is potential clinical utility for identifying malignancy with higher certainty on FNA if such testing allows better preoperative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. An ATA statement provides some guidelines for surgeons managing patients with indeterminate nodules. However, the adoption of these guidelines in practice and the outcomes associated with them are uncertain.

MOLECULAR MARKERS 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 and 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 **PICOs** were used to select literature to inform this review.

Populations

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

Interventions

The relevant interventions of interest 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.

ThyroSeq v3 Test (CPT 0026U)

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

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)	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
Steward et al (2019)	256 patients (286 nodules) with an indeterminate FNA (Bethesda III, IV,	Multicenter (10 sites) prospective	Central pathology review	Classified as malignant	Cross-sectional	Yes

	or V) and underwent thyroid surgery	validation study		or NIFPT or benign		
Livhits et al (2021)	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; NIFPT: noninvasive follicular thyroid neoplasm with papillary-like features

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)		175			98 (93-100)	81 (72-89)		
Steward et al (2019)	286	57	29 (10%)	30%	93 (86-97)	81 (75-86)	68 (58-76)	97 (93-99)
Livhits et al (2021)	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 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)	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)	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)

Acc: accuracy; 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)	110 indeterminate thyroid nodules	<i>BRAF, KRAS, NRAS, RET/PTC1, RET/PTC3, NTRK1</i>	2	38	95	67	79	77
Ohori et al (2010)	100 patients with 117 atypia or follicular lesions of uncertain significance	<i>BRAF, KRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ</i>	NR	60	100	100	92	93
Beaudenon-Hubregtse et al (2014)	53 nodules with indeterminate or nondiagnostic FNA	<i>BRAF, HRAS, KRAS, NRAS, PAX8-PPARγ, RET-PTC1, RET-PTC3</i>		48	89	81	64	

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 (CPT 81445) and ThyraMIR microRNA Classifier (CPT 0018U)

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).⁵⁰

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 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 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 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, 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 (*miRInform*) with ThyraMIR testing on FNA samples and resulted in a sensitivity of 89%, 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 in 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.

SUMMARY OF EVIDENCE

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and to avoid surgical biopsy or resection, the evidence includes a prospective clinical validity study with the Afirma GSC, a systematic review of prospective and retrospective clinical validity studies, and a chain of evidence to support clinical utility. The relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. In a multicenter validation study, the Afirma GSC was reported to have a high NPV (96%; 95% CI, 90%-99%). These results are consistent with an earlier study on the Afirma GSC in the same study population and a randomized controlled trial of Afirma GSC in a similar 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, but the exact NPV is unknown. The available evidence suggests that the decisions a physician makes regarding surgery are altered by Afirma GSC/GSC results; however, it should be noted that long-term follow-up of patients with thyroid nodules who avoided surgery based on GSC results is limited. A chain of evidence can be constructed to establish the potential for clinical utility with GSC testing in cytologically indeterminate lesions, but there is only a single study of the marketed test reporting a true NPV. Clinical input, obtained in 2017, supported the use of the previous version of the Afirma test in FNA of thyroid nodules with indeterminate cytologic findings to rule out malignancy and avoid surgical biopsy with an acceptably low trade-off in missed malignancy. The evidence is sufficient to determine that the technology improves the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule in malignancy and to guide surgical planning, the evidence includes prospective and retrospective studies of clinical validity. The relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. Variant analysis has the potential to improve the accuracy of an equivocal FNA of the thyroid and may play a role in preoperative risk stratification and surgical planning. Single-center studies have suggested that testing for a panel of genetic variants associated with thyroid cancer may allow for the appropriate selection of patients for surgical management for the initial resection. Prospective studies in additional populations are needed to validate these results. Although the presence of certain variants may predict more aggressive malignancies, the management changes that would occur as a result of identifying higher risk tumors, are not well-established. Clinical input, obtained in 2017, considered ThyraMIR microRNA/ThyGenX, Afirma BRAF after Afirma GSC, and Afirma MTC after Afirma GSC to provide a clinically meaningful improvement for patients with cytologic findings suspicious for malignancy to guide surgical planning for the initial resection. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and avoid surgical biopsy or to rule in malignancy for surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq test, a systematic review of retrospective and prospective studies, and 2 retrospective clinical validation studies that used a predicate test 17-variant panel (miRInform) test to the current ThyGenX and ThyraMIR. The relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. In a retrospective validation study on FNA samples, the 17-variant panel (miRInform) test and ThyraMIR had a sensitivity of 89%, and an NPV of 94%. A prospective clinical validation study of ThyroSeq v3 reported an NPV of 97% and PPV of 68%. Similarly, a

systematic review including 3 prospective and 3 retrospective clinical validity studies reported an NPV of 92% and PPV of 70%. No studies were identified demonstrating the diagnostic characteristics of the marketed ThyGenX. No studies were identified demonstrating evidence of direct outcome improvements. A chain of evidence for the ThyroSeq v3 test and combined ThyGenX and ThyraMIR testing would rely on establishing clinical validity. Clinical input, obtained in 2017, considered ThyroSeq v2 to provide a clinically meaningful improvement for patients with indeterminate cytologic findings to rule out malignancy and avoid surgical biopsy and in patients with cytologic findings suspicious for malignancy to guide surgical planning for the initial resection. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

CLINICAL INPUT

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 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 fine needle aspirate (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:

- Afirma Gene Expression Classifier; or
- ThyroSeq v2

For individuals who have fine needle aspirate (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:

- ThyroSeq v2;
- ThyraMIR microRNA/ThyGenX;
- Afirma BRAF after Afirma Gene Expression Classifier; or
- 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.

SUPPLEMENTAL INFORMATION

PRACTICE GUIDELINES AND POSITION STATEMENTS

American Association of Clinical Endocrinologists et al

In 2016, the American Association of Clinical Endocrinologists, American College of Endocrinology, and Association Medical Endocrinology updated their joint guidelines and made the following statements on molecular testing for cytologically indeterminate thyroid nodules: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.”
- “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 (GSCs) for cytologically indeterminate nodules.”

For the role of molecular testing for deciding extent of surgery the following recommendations were made:

- “Currently, with the exception of mutations such as *BRAF* V600E 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 (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

In 2016, the American Thyroid Association (ATA) updated its guidelines on the management of thyroid nodules and differentiated thyroid cancer in adults.⁶⁰ These guidelines make the following statements on molecular diagnostics in thyroid nodules that are atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) on cytology (see Table 11):

Table 11. Molecular Diagnostics in Thyroid Nodules That Are AUS or FLUS or FN or SFN 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 [fine needle aspirate] 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 evidence.

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 (NCCN) guidelines on the treatment of thyroid cancer (V.2.2023) make the following comments about the use of molecular diagnostics in thyroid cancer:⁶¹ For thyroid nodules evaluated with FNA, molecular diagnostics may be employed in the following cases (category 2B recommendation):

- Follicular or oncocytic neoplasm
- Atypia of undetermined significance/Follicular lesion of undetermined significance

The guidelines state that molecular diagnostics have not performed well historically for oncocytic carcinoma. The guideline also endorses the American Thyroid Association and American College of Radiology recommendations for nodule surveillance, described previously above.

Ongoing and Unpublished Clinical Trials

Some currently 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
NCT02947035	Molecular Testing to Direct Extent of Initial Thyroid Surgery	90	Feb 2023
NCT02681328	Randomized Trial Comparing Performance of Molecular Markers for Indeterminate Thyroid Nodules	300	Dec 2024

Unpublished			
NCT03170804	Genomic profiling of nodular thyroid disease and thyroid cancer	200	Jan 2020

NCT: national clinical trial.

Government Regulations

National/Local:

Palmetto GBA determines coverage and reimbursement for laboratories that perform molecular diagnostic testing and submit claims to Medicare in Medicare Jurisdiction E (California, Nevada, and Hawaii). Palmetto GBA's decisions apply for all molecular diagnostic tests for Medicare.

Palmetto GBA has completed an assessment of the Afirma GSC and determined that the test meets criteria for analytic and clinical validity, and clinical utility as a reasonable and necessary Medicare benefit.⁶² Effective January 1, 2012, Palmetto GBA will reimburse Afirma services for patients with the following conditions:

- Patients with one or more thyroid nodules with a history or characteristics suggesting malignancy such as:
 - Nodule growth over time
 - Family history of thyroid cancer
 - Hoarseness, difficulty swallowing or breathing
 - History of exposure to ionizing radiation
 - Hard nodule compared with rest of gland consistency
 - Presence of cervical adenopathy

OR

- Patients who have an indeterminate follicular pathology on fine needle aspiration

Medicare will cover the Afirma GSC test for all Medicare members meeting inclusionary guidelines, no matter which jurisdiction the Medicare member is enrolled in.

MolDX expects this test will be performed once per patient lifetime. Should the unlikely situation of a second, unrelated thyroid nodule with indeterminate pathology occur, coverage may be considered upon appeal with support documentation.

Noridian Healthcare Solutions (L35396) also has an LCD addressing Afirma GSC with the same above criteria. Effective 12/13/2020.

Thyroid:

- BRAF – DX + PRED
- KRAS – PRED for Selumetinib
- HRAS – PRED for Selumetinib
- NRAS – PRED for Selumetinib
- PIK3CA – PRED
- RET – DX
- PAX8/PPARG- DX

ThyraMIR Thyroid miRNA classifier (aPCR based microRNA gene expression classifier) (PRED) evaluates the expression levels of 10miRNA genes within an FNA biopsy: miR-29b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551b-3p.

Oncology Thyroid, provides gene expression analysis of 142 genes utilizing fine needle aspirate, algorithm reported as a categorical result.(AFIRMA – PRED).

ThyraMIR is used as a companion test to ThyGeNEXT when ThyGeNEXT results are inconclusive.

- ThyraMIR, ThyGeNEXT and AFIRMA services will be considered reasonable and necessary for patients with any of the following conditions:An indeterminate pathology on fine needle aspiration
- Patients with one or more thyroid nodules with a history or characteristics suggesting malignancy such as:
 - Nodule growth over time
 - Family history of thyroid cancer
 - Hoarseness, difficulty swallowing or breathing
 - History of exposure to ionizing radiation
 - Hard nodule compared with rest of gland consistency
 - Presence of cervical adenopathy
- RosettaGX Reveal thyroid MicroRNA test, an assay used for the classification of indeterminate thyroid nodules, will be considered reasonable and necessary when the conditions outlined above for ThyraMIR, ThyGeNEXT and AFIRMA are met.
- ThyroSeq is a test utilized to better define the need for thyroid surgery and the type of such surgery. ThyroSeq will be considered reasonable and necessary when the conditions outlined above for ThyraMir, ThyGeNEXT and AFIRMA are met.

(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)

Related Policies

- Genetic Cancer Susceptibility Panels Using Next Generation Sequencing
- Genetic Testing to Determine the Prognosis of Breast Cancer Patients
- Genetic Testing: Mass Spectrometry Based Proteomic Profiling to Determine Treatment for Non-Small-Cell Lung Cancer (NSCLC), e.g., VeriStrat®

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The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through September 2023, the date the research was completed.

Joint BCBSM/BCN Medical Policy History

Policy Effective Date	BCN Signature Date	BCBSM Signature Date	Comments
9/1/14	6/17/14	6/23/14	Joint policy established
1/1/16	10/13/15	11/5/15	Routine maintenance
3/1/17	12/13/16	12/13/16	<p>Added code 81545, deleted NOC code. Updated Hayes information, references and rationale section. Medical Policy Statement changed: The use of the Afirma gene expression classifier in fine needle aspirates of the thyroid that are cytologically considered to be indeterminate (follicular lesion of undetermined significance or follicular neoplasm) may be established in patients who have the following characteristics:</p> <ul style="list-style-type: none"> • Thyroid nodules without strong clinical or radiological findings suggestive of malignancy • In whom surgical decision-making would be affected by test results
3/1/18	12/12/17	12/12/17	Updated rationale section. Added information on FNA when receiving combined ThyGenX and ThyraMIR testing. Added references 41, 46, 50, 51. No change in policy statement.
9/1/18	7/9/18	6/21/18	<ul style="list-style-type: none"> – Added CPT codes 81445, 0018U, 0026U and 81479 – MPS changed to include coverage for ThyraMIR and ThyGenX, ThyroSeq – Added section on RosettGX Reveal test, this is excluded from coverage. – Added clinical input section, updated rationale section and added references 23 and 26-30.

9/1/19	6/18/19		Reformatted rationale section, added references 4, 21, 27, 28, 30, 43, 47, 63 and 65. Added single-gene TERT testing as E/I testing.
1/1/20	10/15/19		Rationale updated, added references 19, 33, 35, 43, 51-54. Removed 8 references. ThyGeNEXT added to MPS replacing ThyGenX.
1/1/21	12/11/20		Rationale updated, added references 26 and 27. MPS reworded, intent remains the same. Code 81210 added as established code. No change in policy status.
1/1/22	10/19/21		Rationale updated, added references #21 and 51. No change in policy status. Added codes 81546, 0208U added as established and 0245U as E/I. Code 81545 deleted.
1/1/23	10/18/22		Code 0245U moved to established section. Routine policy maintenance, added references #19 and #30. No change in policy status.
1/1/24	10/17/23		Rationale updated, references 19, 21, 25 and 34 added. No change in policy status. Vendor managed: N/A (ds)

Next Review Date: 4th Qtr. 2024

BLUE CARE NETWORK BENEFIT COVERAGE
POLICY: GENETIC TESTING- MOLECULAR MARKERS IN FINE NEEDLE ASPIRATES (FNA)
OF THE THYROID

I. Coverage Determination:

Commercial HMO (includes Self-Funded groups unless otherwise specified)	Covered, criteria apply
BCNA (Medicare Advantage)	See government section
BCN65 (Medicare Complementary)	Coinsurance covered if primary Medicare covers the service.

II. Administrative Guidelines:

- The member’s contract must be active at the time the service is rendered.
- Coverage is based on each member’s certificate and is not guaranteed. Please consult the individual member’s certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member’s PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT – HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.