Molecular Markers in Fine Needle Aspirates of the Thyroid

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DESCRIPTION

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

Thyroid nodules are approximately four times more common in women than in men. Palpable nodules increase in frequency throughout life, reaching a prevalence of about 5% in the U.S. population for individuals aged 50 years and older having palpable thyroid nodules. Nodules are even more prevalent when the thyroid glands are examined at autopsy or surgery, or when using ultrasonography; 50% of thyroids studies have nodules, which are almost always benign. New nodules develop at a rate of about 0.1% per year, beginning in early life, but they develop at much higher rate (approximately 2% per year) after exposure to head and neck irradiation.

By contrast thyroid carcinoma is uncommon. For the U.S. Population, the lifetime risk of being diagnosed with thyroid carcinoma is 1.2%. Thyroid carcinoma is currently the fifth most common malignancy diagnosed in women. The disease is also diagnosed more often in white North Americans than in African Americans. The main histologic types of thyroid carcinoma are: differentiated (including papillary, follicular, and Hurthle cell); medullary; and anaplastic which is an aggressive undifferentiated tumor. In 2022 there will is an estimated 43,800 new cases that will be diagnosed with thyroid cancer and estimated deaths of 2,230.

Evaluating all nodules for malignancy is difficult, because benign nodules are so prevalent and because thyroid carcinoma is so uncommon. Fine needle aspiration (FNA) with ultrasound guidance is the procedure of choice for evaluating suspicious thyroid nodules and distinguish benign thyroid lesions and 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. However, the remaining 20% to 30% have equivocal findings (inconclusive, indeterminate, atypical or suspicious), usually due to overlapping cytologic features between benign and malignant nodules; these nodules usually require surgery for final diagnosis.

Thyroid FNA cytology is classified according to Bethesda System for Reporting Cytopathology: Recommended Diagnostic Categories, see below:

The Bethesda System for Reporting Cytopathology: Recommended Diagnostic Categories

Risk Category	Definition	Diagnostics
I	Non-diagnostic or Unsatisfactory	Cyst fluid only Virtually acellular specimen Other (obscuring blood, clotting artifact, etc.)
II	Benign	Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc.) Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context Consistent with granulomatous (subacute) thyroiditis Other

Risk Category	Definition	Diagnostics
III	Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance	
IV	Follicular Neoplasm or Suspicious for a Follicular Neoplasm	Specify if Hurthle cell (oncocytic) type
V	Suspicious for malignancy	Suspicious for papillary carcinoma Suspicious for medullary carcinoma Suspicious for metastatic carcinoma Suspicious for lymphoma Other
VI	Malignant	Papillary thyroid carcinoma Poorly differentiated carcinoma Medullary thyroid carcinoma Undifferentiated (anaplastic carcinoma) Squamous cell carcinoma Carcinoma with mixed features (specify) Metastatic carcinoma Non-Hodgkin lymphoma Other

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 the diagnosis. Approximately 80% of patients with indeterminate cytology undergo surgical resection; postoperative evaluation has revealed a malignancy rate ranging from 6% to 30%, making this a clinical process with very low specificity. Thus, if 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 vs total or subtotal thyroidectomy with or without lymph node dissection) depending on several factors, including histologic subtype and risk-stratification strategies (tumor size, patient age). If a diagnosis cannot be made intraoperatively, a lobectomy is typically performed, and if on postoperative

histology the lesion is malignant, a second surgical intervention may be necessary for completion thyroidectomy.

The Bethesda System for Reporting Cytopathology: Implied Risk of Malignancy and Recommended Clinical Management

Diagnostic Category	Risk of Malignancy (%)	Usual Management (actual management may depend on other factors (e.g. clinical, sonographic) besides FNA interpretation)
Non-diagnostic or Unsatisfactory	1-4	Repeat FNA with ultrasound guidance
Benign	0-3	Clinical follow up
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance	5-15 (estimate extrapolated from histopathologic data from patients with "repeated atypicals"	Repeat FNA
Follicular Neoplasm or Suspicious for a Follicular Neoplasm	15-30	Surgical lobectomy
Suspicious for Malignancy	60-75	Near-total thyroidectomy or surgical lobectomy (in the case of "suspicious for metastatic tumor" or a "malignant" interpretation indicating metastatic tumor rather than a primary thyroid malignancy, surgery may not be indicated).
Malignant	97-99	Near-total thyroidectomy(in the case of "suspicious for metastatic tumor" or a "malignant" interpretation indicating metastatic tumor rather than a primary thyroid malignancy, surgery may not be indicated).

FNA: fine need aspiration

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 pre-existing well-differentiated papillary or follicular carcinomas. Medullary thyroid carcinoma originates from parafollicular or C cells, and accounts for about 3% of all thyroid cancers.

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

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

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

Genetic Variants Associated with Thyroid Cancer
Various genetic variants have been discovered in thyroid cancer. The 4 gene mutations that are most common and carry the highest impact on tumor diagnosis and prognosis are BRAF and RAS single-nucleotide variants (SNVs) and RET/PTC and PAX8/PPARy rearrangements.

Papillary carcinomas carry single-nucleotide variants (SNVs) 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 SNVs are highly specific for PTC. Follicular carcinomas harbor either RAS SNVs or PAX8/PPARy rearrangement. These mutations identified in 70% to 75% of follicular carcinomas. Genetic alterations involving PI3K/AKT signaling pathway also occur in thyroid tumors, although they are rare in well differentiated thyroid cancers and have higher prevalence in less differentiated thyroid carcinomas. Additional variants known to occur in poorly differentiated and anaplastic carcinomas involve the TP53 and CTNNB1 genes.

Medullary carcinomas, which can be familial or sporadic, frequently possess SNVs located in the RET gene.

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

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. 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. Also, the co-occurrence of BRAF or RAS variants with TERT or TP53 variants may identify a subset of thyroid cancers with unfavorable outcomes.

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

The ThyroSeq v2 Next Generation Sequencing Panel (CBLPath, Ocala, FL) is a NGS sequencing panel of more than 60 genes. According to the CBLPath'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. In particular, it has been evaluated in patients with follicular neoplasm and/or suspicious for follicular neoplasm on FNA as a test to increase both sensitivity and specificity for cancer diagnosis.

The ThyroSeq v3 Next Generation Sequencing Panel of DNA and RNA is expanded to analyze 112 genes, providing information on > 12,000 mutation hotspots and > 120 gene fusion types. The test detects 4 classes of genetic alterations: 1) mutations (SNVs, indels); 2) gene fusions; 3) gene expression alterations; and 4) copy number variations (CNVs). The test utilizes a proprietary Genomic Classifier (GC) based on the algorithmic analysis of all detected genetic alterations to report the test as positive or negative.

The ThyGenX Thyroid Oncogene Panel (formerly miRInform Thyroid; Interpace Diagnostics, Parsippany, NJ) is a next-generation 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.

ThyGeNEXT (Interpace Diagnostics) is a thyroid cancer mutational panel using next generation sequencing (NGS) simultaneously analyzes more than 150 genetic alterations associated with papillary and follicular thyroid carcinomas, the two most common forms of thyroid cancer. The ThyGeNEXT panel builds on the company's ThyGenX panel (launched in August 2014) by adding numerous molecular markers, gene mutations, and RNA fusions, resulting in a more comprehensive set of indicators to identify malignant or benign nodules and ascertain aggressiveness and other characteristics.

TERT (telomerase reverse transcriptase) promoter mutations (Interpace Diagnostics, Parsippany, NJ) is a molecular marker predictor of aggressiveness of thyroid cancer. Currently, the ThyGenX mutation panel includes the following markers that are predictive of thyroid cancer from cytologically indeterminate thyroid nodules: BRAF, HRAS, KRAS, NRAS, RET/PTC, PAX8/PPARy and PIK3CA. By adding TERT, the ThyGenX panel will be a strong predictor of thyroid cancer but will also provide evidence that a positive result indicates that the cancer is likely to be more aggressive in nature.

Gene Expression Profiling

Genetic alterations associated with thyroid cancer can be assessed using 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.

Afirma Gene Expression Classifier (GEC) (Afirma GEC; 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 ("rule-out") who are at low risk for cancer.

The Afirma Genomic Sequencing Classifier (Afirma GSC; Veracyte, South San Francisco, CA) is a second-generation test that combines ribonucleic acid (RNA) sequencing using a proprietary algorithm to analyze the expression of different genes in thyroid FNA specimens in order to classify indeterminate thyroid nodules. The Afirma Genomic Sequencing Classifier is described as a "rule out" test because a negative (benign) result rules out the presence of cancer.

ThyraMIR (Interpace Diagnostics, Parisippany, N.J.) is a microRNA expression-based classifier intended for use in thyroid nodules with indeterminate cytology. There are 10 microRNAs evaluated. ThyrMIR may be offered alone or in combination with a thyroid

mutation panel (ThyGenX/ThyGeNEXT) to purportedly enhance specificity and sensitivity testing results.

RosettaGX Reveal (Rosetta Genomics, Philadelphia, PA) is a microRNA expression-based classifier to differentiate indeterminate thyroid nodules as benign, suspicious for malignancy or as having high risk for medullary carcinoma (an aggressive form of thyroid cancer). RosettaGX Reveal can be performed using the existing FNA smears from routinely prepared cytology slides from the patient's initial biopsy. There are 24 microRNAs evaluated.

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

Algorithmic Testing Using Afirma GEC with Afirma MTC and Afirma BRAF
In addition to Afirma GEC (e.g., Afirma Genomic Sequencing Classifier [GSC]), Veracyte also markets 2 "malignancy classifiers" that use mRNA expression-based classification to analyze thyroid nodules that have been classified as malignant or suspicious for malignancy through cytopathology or as suspicious for malignancy on GSC. Afirma MTC was developed to identify the presence of medullary thyroid cancer (MTC) while Afirma BRAF was designed to determine the presence of BRAF V600E mutation. Afirma MTC and Afirma BRAF are included in Afirma GSC.

Afirma MTC and Afirma BRAF Testing Algorithm

Test 1	Test 1 Result	Reflex to Test 2
Thyroid nodule on fine needle aspirate	"Intermediate"	Afirma MTC
Afirma GEC	"Malignant" or "Suspicious"	Afirma MTC
Afirma GEC	"Suspicious"	Afirma BRAF

In a description of the Afirma BRAF test, the following have been proposed as benefits of the mRNA-based expression test for BRAF variants:

1. PCR based methods may have low sensitivity, requiring that a large proportion of the nodule have a relevant variant;
2. Testing for only 1 variant may not detect patients with low frequency variants that result in the same pattern of pathway activation; and
3. PCR-based approaches with high analytic sensitivity may require a large amount of DNA that is difficult to isolate from small FNA samples.

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 hemi vs a total thyroidectomy or performance of a central neck dissection.

Algorithmic Testing Using Afirma Xpression Atlas (Afirma XA)
The Afirma Xpression Atlas (Afirma XA) is a recently introduced add-on test that provides physicians with genomic alteration information from the same FNA samples that are used in Afirma GSC testing. This test may enable physicians to tailor surgery strategy or treatment options for patients whose thyroid nodules are cancerous or suspicious for cancer. The RNA sequencing based test measures 761 DNA variants (including the most common and emerging variants associated with thyroid carcinoma such as: BRAF, DICER1, EIF1AX, H/K/N-RAS, RET, TP53, TG, ZFHX3) and 130 RNA fusions (including the most common and emerging fusions associated with thyroid carcinoma such as: ALK, BRAF, NTRK, PAK8, RET) in over 500 genes that are linked to thyroid cancer. Afirma XA also detects variants and fusions that may inform targeted therapy such as ALK, BRAF, EGFR, MET, NTKR, PAX8/PPAR-y, RAS, RET, ROS1.

Algorithmic Testing Using ThyGenX and ThyraMIR
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 (negative predictive value) of the second test. Patients with a negative result from the ThyraMIR test would be followed with active surveillance and avoid a surgical biopsy.

The ThyGeNEXT panel builds on the company's ThyGenX panel (launched in August 2014) by adding numerous molecular markers, gene mutations, and RNA fusions, resulting in a more comprehensive set of indicators to identify malignant or benign nodules and ascertain aggressiveness and other characteristics.

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 is to rule out malignancy and eliminate the need for surgical resection. 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 after FNA of thyroid nodule presently proceed to surgical biopsy or surgical resection. The potential beneficial outcome of primary interest would be avoiding an unneeded surgical biopsy or resection (e.g., lobectomy or hemithyroidectomy) in a true-negative thyroid nodule that is benign. A potential harmful outcome is those resulting from a false-negative testing result, 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 result in worsening of health outcomes.

Afirma Gene Expression Classifier (GEC)/Afirma Genomic Sequencing Classifier (GSC)

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

Chudova et. al. (2010) described the development and initial clinical validation of a version of the Afirma GEC. The classifier was trained on 178 retrospectively identified surgical thyroid specimens, which represented a variety of malignant and benign disorders, and separately on a set of 137 FNA samples with known surgical pathology. The classifier was developed with the objective of achieving a NPV (negative predictive value) specificity of 95% and specificity of 70%. The tissue-trained classifier was tested on an independent sample of 48 FNAs (24 with indeterminate cytopathology, 24 with a mix of malignant and benign cytopathology). The FNA trained classifier was separately tested on the same sample of 48 FNAs. In the 24 samples with indeterminate cytopathology, sensitivity and specificity were 100% (95 confidence interval (CI), 64% to 100%) and 73.3% (95% CI, 49% to 89%), respectively.

Prospective Clinical Validation
Alexander et. al. (2012) reported on a 19-month, prospective, multicenter (49 academic and community) sites, study of the Afirma GEC. 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.

The GEC used in the Alexander et. al. study were retrained on a set of 468 samples, comprised of 220 banked tissue samples, 14 ex vivo operative FNA samples, and 234 prospective clinical FNA samples described above.

After exclusion of the 25 used for test validation and those that did not have a valid GEC result, 265 FNA samples were evaluated with Afirma GEC. Of the 265 samples, 85 were malignant; the GEC correctly identified 78 of the 85 as suspicious (92% sensitivity; 95% CI, 84% to 97%). Specificity was 52% (95% CI, 44% to 59%). NPV (negative predictive value) ranged from 85% for “suspicious cytologic findings” to 95% for “atypia of undetermined clinical significance.” There were 7 FNAs with false-negative results, 6 of which were thought to be due to hypocellular aspirate specimens.

Retrospective Clinical Validation
In 2014, Alexander et. al. reported results from a multicenter retrospective analysis of 339 thyroid nodules that underwent Afirma GEC testing for indeterminate cytology on FNA (follicular lesion of undetermined significance/atypia of undetermined significance,

follicular neoplasm, or suspicious for malignancy) at 5 academic medical centers. Most nodules sent for GEC testing were follicular lesions of undetermined significance/atypia of undetermined significance or follicular neoplasm. The distribution of GEC testing results for each cytologic classification is shown below.

GEC Testing Results for Alexander et. al. (2014)

Cytologic Classification	Total, n	GEC Testing Results, n(%)
Atypia or Follicular lesion of undetermined significance	165	Benign 91 (55%), Suspicious 66 (40%), Non-diagnostic 8 (5%)
Follicular neoplasm	161	Benign 79 (49%), Suspicious 73 (45%), Non-diagnostic 9 (6%)
Suspicious for malignancy	13	Benign 4 (31%), Suspicious 9 (69%), Non-diagnostic 0
Total	339	Benign 174, Suspicious 148, Non-diagnostic 17

A subset of patients whose nodules underwent GEC testing had a subsequent thyroid resection. Among 148 cases with suspicious Afirma GEC findings, surgery (thyroid resection) was recommended for 141 (95%). For the 174 cases with benign Afirma GEC findings, surgery was recommended for 4 (2%; $p<0.01$). Using the assumption that, absent the GEC results, thyroid surgery would be recommended for patients with cytologically indeterminate FNA results, the authors reported that the GEC results altered management in 50% of patients. The below table shows thyroidectomy biopsy results for the subset of patients shown in the table above who underwent surgery.

Thyroidectomy Results for Alexander et. al. (2014)

GEC Results	Total, n	Surgery recommended, n	Surgery Completed, n	Pathology Malignant, n (%)
Suspicious	148	141	121	53 (44% of those with completed surgery)
Benign	174	4	11	1 (9% of those with completed surgery)

Seventeen patients who had indeterminate cytology, benign Afirma GEC results, and did not undergo surgery had follow-up beyond 1 year. Of those, 3 patients underwent surgical removal of the nodule because of compressive symptoms (n=2) or nodule growth

(n=1); all nodules were benign on final histology. The remaining 14 patients had ongoing follow-up with ultrasound with no ongoing evidence of malignancy. The study demonstrated site-to-site variation in the proportion of samples that were GEC benign. A benign GEC result did not completely rule out malignant pathology. Long-term follow-up was available for only a small proportion of patients with benign GEC findings who did not undergo surgery.

In 2016, Santhanam et. al. reported results of a meta-analysis of studies reporting on the performance of the Afirma GEC in cytologically indeterminate nodules. Seven studies met the inclusion criteria, which required that studies reported on the use of the Afirma GEC in nodules that were indeterminate on FNA (including atypia of undetermined significance or follicular lesion of undetermined significance; suspicious for follicular/Hurthle cell neoplasm; suspicious for malignancy), and thyroidectomy was performed as a reference standard in at least the cases where the index test was suspicious. All studies were judged to be at low risk of bias for patient selection, and most for GEC test selection, whereas the risk of bias in the final histopathology was low in 3 studies, unclear in 3 studies, and high in 1 study. In the pooled cohort, the prevalence of malignancy was 37.1%. The main results of the analysis are summarized below.

Pooled GEC Performance for Santhaname et. al (2016)

Outcomes	Point Estimate	95% Confidence Interval	I^2
Sensitivity	95.7%	92.2% to 97.9%	45.4%
Specificity	30.5%	26.0% to 35.3%	92.1%
Positive likelihood ratio	1.20	0.996 to 1.44	
Negative likelihood ratio	0.2	0.11 to 0.36	
Diagnostic odds ratio	7.9	4.1 to 15.1	

Retrospective single center studies, including Harrell and Bimston (2014), Lastra et. al. (2014), McIver et. al. (2014), Yang et. al. (2016) has reported the diagnostic accuracy of the Afirma GEC, are summarized in the table below. These 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 assays impossible. However, the rates of malignancy among patients with Afirma benign results who did undergo surgery are consistently low. One exception is the study by Harrell and Bimston (2014); it may be reflective of a higher-than-usual overall rate of malignancy in patients with indeterminate FNA results.

Single-Center Studies Reporting Afirma GEC Results

Study (Year)	Population of Indeterminate FNA Samples	Afirma Test Result	N	N with Thyroidectomy	N with Malignancy on Thyroidectomy

Harrell and Bimston (2014)	58 FLUS/AUS or FN	Suspicious Benign	36[a] 20	30 5	21 2
Lastra et. al. (2014)	69 (51.5%) FLUS/AUS 39 (29.5%) FN 25 (19%) FNOF	Suspicious Benign	62 70	48 2	22 0
McIver et. al. (2014)	12 (11.4%) FLUS/AUS 93 (88.6%) FN/HCN	Suspicious Benign	44[b] 16	32 4	5 1
Yang et. al. (2016)	165 (76%) FLUS/AUS 24 (11%) SFN/FN	Suspicious Benign	80 94	62 5	32 0
Witt et. al. (2016)	47 FLUS/AUS or SFN/FN (32 with GEC attempted[c])	Suspicious Benign	15 14	15 0	6 Not applicable: followed clinically

AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspirates; FNOF: follicular neoplasm with oncocytic features; HCN: Hurthle cell neoplasm; SFN: suspicious for follicular neoplasm.

[a] Two samples inadequate due to low mRNA content

[b] GEC results were available for 60 subjects

[c] Three samples were inadequate

There are limited data on the true negative rate of individuals with indeterminate FNA cytology and Afirma GEC benign results. Supportive information on the accuracy Afirma GEC benign results can be obtained from studies that report on long-term follow up of individuals with indeterminate FNA cytology and Afirma GEC benign results. Angell et. al. (2015) retrospectively compared clinical outcomes for individuals with indeterminate FNA cytology and Afirma GEC benign results with individuals to cytologically benign nodules. A total of 95 cytologically indeterminate/Afirma GEC benign nodules in 90 patients were compared with 1224 cytologically benign nodules identified from a single center, prospectively collected database. Five nodules in the cytologically indeterminate were resected; of the remaining 90 nodules, 58 (64.4%) had follow-up ultrasound available at a median of 13 months post-diagnosis. When nodule growth was defined by a volume increase of 50% or more, 17.2% cytologically indeterminate/Afirma GEC benign were considered to have grown compared with 13.8% of cytologically benign nodules ($p=0.44$). Surgical resection was more common in cytologically indeterminate/Afirma GEC benign nodules (13.8% vs 0.9%, $p<0.001$).

In 2017, Duh et. al. reported on a updated systematic review of the methods of diagnostic accuracy studies of the Afirma Gene Expression Classifier. Twelve studies evaluated; the most common methodologic flaw was lack of reference standard diagnosis assignment to un-excised GEC-benign ITNs (indeterminate thyroid nodules). Reviewers did not report pooled results because most included studies that did not meet minimal quality standards, primarily due to the lack of reference standard diagnoses for GEC-benign nodules.

Hang et.al. (2017) retrospectively analyzed consecutive thyroid fine-needle aspiration specimens with indeterminate diagnoses on which Afirma gene expression classifier (GEC) was performed. Surgical pathology material was reviewed with the reclassification of nodule into noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). GEC testing was performed on 384 fine-needle aspiration specimens diagnosed as atypia of undetermined significance (AUS) (304 cases) and suspicious for a follicular neoplasm (SFN) (80 cases) and yielded a suspicious result in 152 of the AUS cases (50%) and 50 of the SFN cases (63%). Thyroidectomy was performed on 177 patients. After reclassifying NIFTP, the positive predictive value of GEC decreased from 42% (95% confidence interval [95% CI], 39%-45%) to 24% (95% CI, 22%-26%) in the AUS group and from 23% (95% CI, 19%-27%) to 13% (95% CI, 9%-18%) in the SFN group. Total thyroidectomy was performed more frequently than a partial thyroidectomy in patients with AUS with a suspicious GEC result compared with pre-GEC controls (68% vs 49%; P = .037). The authors concluded, reclassification of NIFTP significantly decreases the positive predictive value of GEC in indeterminate thyroid nodules. Nevertheless, the majority of patients with indeterminate thyroid nodules with a suspicious GEC result in the study institution have undergone total thyroidectomy. This finding raises concerns over reliance on a suspicious GEC result by clinicians to justify total thyroidectomy.

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

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

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 indirect evidence was developed, which addresses 2 key questions:

1. Does the use of Afirma GEC in individual with cytologically indeterminate thyroid nodules lead to changes in management (in this case, reduced thyroid resections)?
2. Do those management changes improve outcomes?

Changes in Management
The clinical setting in which the Afirma GEC is meant to be used is well-defined: individuals with atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) or follicular neoplasm/suspicious for follicular neoplasm on FNA, who do not have other indications for thyroid resection (i.e. in whom the GEC results would play a role in surgical decision making).

Decision impact studies, most often reporting on clinical management changes but not on outcomes after surgical decisions were made, suggest that in a least some cases, surgical decision-making is changed. These studies are described briefly.

Duick et. al. (2012) reported on the impact of Afirma GEC test results on physician and patient decision making to operate on thyroid nodules with indeterminate cytology and Afirma GEC benign results in a sample of 395 nodules from 368 patients. Surgery was performed in 7.6% of the patients with indeterminate cytology and a benign GEC result, less than the historical rate of thyroid resection (74%) in patients with indeterminate cytology.

The 2014 study by Alexander et. al. provides some evidence about clinical management changes for patients with indeterminate thyroid nodules with the use of the Afirma GEC. While the treating physicians presumably elected to obtain the GEC testing with the intent of altering management recommendations, the magnitude of the difference in surgical recommendations for patients with GEC suspicious or benign results was large.

Two studies (Aragon Han et. al. (2014), Noureldine et. al. (2015)) were identified that evaluated the potential for the Afirma GEC to change surgical decision making by comparing actual surgical decision making when the Afirma GEC was used to predict surgical decision making based on a management algorithm. In both, surgical decision making was estimated to change in at least some proportion of patients (approximately 10%-15%).

Sipos et. al. (2016) performed a retrospective study of nonacademic medical practices using the Afirma GEC and determine the long-term nonoperative rate of thyroid nodules with benign results. Of the patients with Afirma "benign" results for 36 months ($\pm$ 3 months) of follow-up, 17.3% underwent surgery. 88% of all surgeries were performed within the first 2 years after a "benign" Afirma GEC result.

Abeykoon et. al. (2016) studied the impact of implementing Afirma GEC at a single center. Surgical recommendations for patients with indeterminate thyroid nodules decreased from 81.5% pre-Afirma GEC to 50% post-Afirma GEC. The rate of malignant

surgical pathology diagnosis increased from 20% pre-Afirma GEC to 85.7% post-Afirma. The implementation of Afirma GEC decreased the number of surgical recommendations and increased the rate of malignancy detected for patients who received a surgical biopsy.

Chaudhary et. al. (2016) studied the impact on surgical outcomes pre- and post-implementation of Afirma GEC. A total of 158 FNAs were sent for Afirma GEC with 73 "suspicious" and 8 "benign" Afirma cases going through surgeries. Compared with before implementation of Afirma GEC, the rate for surgical biopsy decreased from 61% to 54% but was not statistically significant. In the SFN, the rate of surgical biopsy significantly decreased from 76% to 52%.

Dhingra et. al. (2016) studied the effects of a FNA protocol combining the expert thyroid cytopathology and Afirma GEC in a community practice. Historical data was compared with data after implementation of the FNA protocol. Prior to implementation of the FNA protocol, the rates of indeterminate cytology and diagnostic surgery were 26% and 24%. After implementation of the FNA protocol, the rates of indeterminate cytology and diagnostic surgery decreased to 10% and 6%. The effect of Afirma GEC implementation could not be ascertained given the FNA protocol combining expert thyroid cytopathology and Afirma GEC used in the study.

Improved Outcomes

A simplified decision model was developed for use with Afirma GEC in individuals with cytologically indeterminate FNA samples. It is assumed that when Afirma GEC is not used, patients with cytologically indeterminate FNA results undergo thyroid resection. When Afirma GEC is used, those with Afirma suspicious lesions undergo resection, while those who have Afirma benign lesions do not. In this case, compared with 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 the rate of cancer in cytologically indeterminate thyroid nodules is approximately 20%, 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), 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.

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 amendable 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.

At present, the existing standard of care for thyroid nodules is based on intervention that is stratified by FNA cytology results, which are grouped into categories with differing prognosis. Avoiding an invasive surgery in situations where patients are at very low likelihood of having an invasive tumor is likely beneficial, given the small but potentially significant adverse events associated with thyroidectomy or hemithyroidectomy. Among the low-risk population, the alternative to surgical biopsy is ongoing active surveillance.

In 2019, Harrell et. al. completed a statistical comparison of the Veracyte Afirma Gene Expression Classifier (GEC) and Afirma Genomic Sequence Classifier (GSC) in a community endocrine surgical practice. The Veracyte Afirma Gene Expression Classifier (GEC) has been the most widely used negative predictive value molecular classifier for indeterminate cytology thyroid nodules since January 2011. To improve the specificity and further reduce unnecessary thyroid surgeries, a second-generation assay (Afirma Genetic Sequence Classifier [GSC]) was released for clinical use in August 2017. The authors reported 11 months of clinical outcomes experience with the GSC and compare them to our 6.5-year experience with the GEC. They searched their practice registry for fine needle aspiration biopsy (FNAB) nodules with Afirma results from January 2011 through June 2018. GEC versus GSC results were compared overall, in oncocytic and nononcocytic aspirates and by pathologic outcomes. GSC identified less indeterminate cytology nodules as suspicious (38.8%; 54/139) when compared to GEC (58.4%; 281/481). There was a decrease of in the percentage of oncocytic fine-needle aspiration thyroid biopsy (FNAB) subjects classified as suspicious in the GSC group, with 86 of 104 oncocytic indeterminates (82.7%) classified as suspicious by GEC and 12 of 34 (35.3%) classified as suspicious by GSC. The surgery rate in patients with oncocytic aspirates fell from 56% in the GEC group to 31% in the GSC-evaluated group (45%). Pathology analysis demonstrated a false-negative percentage for an incomplete surgical group of 9.5% for GEC and 1.2% for GSC. The authors concluded GSC data suggests that the GSC further reduces surgery in indeterminate thyroid nodules by improving the specificity of Afirma technology without compromising sensitivity. A primary determinant for this change is a significant improvement in the specificity of the Afirma GSC test in oncocytic FNAB aspirates.

RosettaGX Reveal

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

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 the table below:

RosettaGX Reveal Performance

Outcomes	Value	95% Confidence Interval
Samples passing QC (n=189)		
Sensitivity %	85	74 to 93
Specificity %	72	63 to 79
Negative Predictive value	91	84 to 96
Samples with 3 Pathologists agreeing on final diagnosis (n=150, subset of samples passing QC)		
Sensitivity %	98	87 to 100
Specificity %	78	69 to 85
Negative predictive value	99	94 to 100

In 2018, Walts et. al. performed a retrospective analysis of the performance of the RosettaGX Reveal thyroid miRNA and the Afirma Gene Expression Classifiers (AGEC) in a cohort of cytologically indeterminate thyroid nodules. Eighty-one samples (54 Bethesda III, 26 Bethesda IV, 1 Bethesda V) with available AGEC (74 AGEC-SUSP and 7 AGEC-BENIGN) and surgical pathology results were studied from three academic centers. Reveal was performed in a blinded fashion. The final diagnoses were benign/NIFTP (n = 63) and malignant (n = 18). The overall "correct" rate was 64.2% for Reveal and 28.4% for AGEC (P = 1.4e-6). The specificity of Reveal was 60.3%, compared with 9.5% for AGEC (P = 2.1e-9). Among the 18 malignant cases, 77.8% and 94.4% were correctly classified as suspicious by Reveal and AGEC, respectively (P = 0.2). In the FLUS and the FN group, the specificity of AGEC was lower than the specificity of Reveal. Whether the 7 NIFTP in our study were considered benign or malignant, specificity and PPV of Reveal were higher than those of AGEC. Reveal also outperformed AGEC in correctly classifying the 26 benign Hurthle lesions studied (P = 7.6e-5). The authors concluded, Reveal outperformed AGEC in this cohort, whether NIFTP is considered benign or malignant, and in Hurthle lesions. Reveal has the potential to reduce the number of unnecessary resections in patients with indeterminate thyroid cytology. Based on their findings and the practical advantages offered by Reveal methodology, large prospective studies are warranted.

No prospective clinical studies for RosettaGX Reveal were identified.

Clinically Useful

No evidence directly demonstrating improved outcomes in patients managed with RosettaGX Reveal was identified.

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.

Summary
In a single multicenter validation study, the Afirma GEC test has been reported to have a high NPV (range 90%-95%). These results are supported by an earlier development and clinical validation study (Chudova et. al.), but the classifiers used in the 2 studies do not appear to be identical. In an additional multicenter and multiple single-center studies, there is suggestive evidence that rates of malignancy are low in Afirma benign patients, but the exact NPV is unknown. The available evidence has suggested that physician decision making about surgery is altered by GEC results, although long term follow up of patients with thyroid nodules who avoided surgery based on GEC results is limited. A chain of evidence can be constructed to establish the potential for clinical utility with Afirma GEC testing in cytologically indeterminate lesions, but with only 1 study marketed test reporting a true NPV, the clinical validity is uncertain. In 2019, Harrell et. al. completed a statistical comparison of the Veracyte Afirma Gene Expression Classifier (GEC) and Afirma Genomic Sequence Classifier (GSC) in a community endocrine surgical practice. The Veracyte Afirma Gene Expression Classifier (GEC) has been the most widely used negative predictive value molecular classifier for indeterminate cytology thyroid nodules since January 2011. To improve the specificity and further reduce unnecessary thyroid surgeries, a second-generation assay (Afirma Genetic Sequence Classifier [GSC]) was released for clinical use in August 2017. The authors concluded GSC data suggests that the GSC further reduces surgery in indeterminate thyroid nodules by improving the specificity of Afirma technology without compromising sensitivity. A primary determinant for this change is a significant improvement in the specificity of the Afirma GSC test in oncocytic FNAB aspirates.

For the RosettaGX Reveal test, 1 analytic validation study and 2 retrospective clinical validation has been reported. No prospective studies for patients managed with the RosettaGX Reveal were identified, so the clinical utility 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 FNA of thyroid nodules is to rule in malignancy and to guide surgical approach or management. The relevant population of interest includes individuals with indeterminate findings on fine needle aspirate(s) of thyroid nodules. Patients with indeterminate findings would presently proceed to surgical biopsy perhaps with intraoperative pathology consultation (i.e., intra-operative frozen section) if available. The relevant intervention of interest is testing for molecular markers single nucleotide variants (SNVs) and gene rearrangements to rule in malignancy and to use molecular marker results that are positive for variant associated with malignancy to guide surgical planning to ensure the capability for intraoperative pathologic confirmation of malignancy in order to be able to adjust to

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

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

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

Gene Expression Classifiers to Predict Malignancy

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

Less evidence exists on the validity of gene expression profiling (specifically, the Afirma BRAF and Afirma MTC tests, Afirma Xpression Atlas [XA] 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.

Afirma BRAF and Afirma MTC
In the Diggans study, 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. 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 the Kloos study (2016) describing the development and validation of the Afirma MTC classifier, the MTC classifier was evaluated in a sample of 10,488 thyroid nodule FNA samples referred to GEC testing (the Afirma GEC described above). In this sample, 43 cases were Afirma MTC positive, of which 42 were considered to be clinically consistent with medullary thyroid carcinoma on pathology or biochemical testing, for PPV of 97.7% (95% CI, 86.2% to 99.9%).

Afirma Xpression Atlas (XA)
Afirma Xpression Atlas (XA) is a recently introduced add-on test, available for Afirma Genomic Sequence Classifier (GSC) suspicious and Bethesda V and VI nodules. Afirma Expression Atlas (XA)s is not a cancer rule-out test. The Afirma Xpression Atlas (XA) findings may predict tissue cellular morphology, clinical syndromes, cancer behavior (including metastasis), prognosis, and facilitate the selection of effective targeted therapy in the appropriate clinical setting.

In 2019, Angell et. al. reported on the analytical and clinical validation of Afirma Xpression Atlas (XA) which detects gene variants and fusions in thyroid nodule FNA samples using whole transcriptome RNA-sequencing. Its intended use is among cytologically indeterminate nodules thar are Afirma GSC suspicious, Bethesda V/VI nodules, or known thyroid metastases. DNA and RNA were purified from the same sample across 943 blinded FNAs and compared by multiple methodologies, including whole-transcriptome RNA-seq, targeted RNA-seq, and targeted DNA-seq. An additional 695 blinded FNAs were used to define performance for fusions between whole-transcriptome RNA-seq and targeted RNA-seq. They quantified the reproducibility of the whole-transcriptome RNA-seq assay across laboratories and reagent lots. Finally, variants and fusions were compared to histopathology results. Of variants detected in DNA at 5 or 20% variant allele frequency, 74 and 88% were also detected by XA, respectively. XA variant detection was 89%when compared to an alternative RNA-based detection method. Low levels of expression of the DNA allele carrying the variant, compared with the wild-type allele, was found in some variants not detected by XA. 82% of gene fusions detected in a targeted RNA fusion assay were detected by XA. Conversely, nearly all variants or fusions detected by XA were confirmed by an alternative method. Analytical validation studies demonstrated high intra-plate reproducibility (89%-94%), inter-plate reproducibility (86–91%), and inter-lab accuracy (90%). Multiple variants and fusions previously described across the spectrum of thyroid cancers were identified by XA, including some with approved or investigational targeted therapies. Among 190 Bethesda III/IV nodules, the sensitivity of XA as a standalone test was 49%. The authors concluded, we have demonstrated clinical and analytical validation of the Afirma XA, which reports variants and fusions from a panel of 511 genes that have been associated with thyroid cancer. This added clinical information is intended to supplement clinical decision making among patients with Bethesda III-VI nodules. Clinicians are reminded that most patients with thyroid cancer have an excellent prognosis, and the greatest impact of this added genomic information may be to facilitate treatment that is less aggressive, rather than more aggressive. The information obtained from variants and fusions assessment may offer new precision medicine insights from diagnostic FNA samples and the opportunity to advance individualized patient care.

No prospective clinical studies for Afirma Xpression Atlas (XA) were identified.

TERT Single-Gene Testing

TERT (telomerase reverse transcriptase) promoter mutations has been proposed for risk stratification and predicting patient outcomes. TERT can be performed as part of a mutation panel (ThyGenX) or on an individual basis. Published data suggests that TERT mutations can extend the life span of the tumor cell and allow time for other mutations to develop. Mutations in the TERT promoter region are found in thyroid cancers and seem to act synergistically when they occur with the BRAF V600 mutation. The coexistence of mutations in TERT and BRAF genes have shown to dramatically increase the risk of thyroid cancer aggressiveness. By adding TERT, the panel (ThyGenX) not only acts as a strong predictor of thyroid cancer, but also provides evidence that a positive result indicates the cancer is more likely to be more aggressive, which enables the physician to make the most informed surgical choice for the patient.

Nikiforov et. al. (2014) evaluated the accuracy of the ThyroSeq v2 next generation sequencing (NGS) panel that includes tests for SNVs in 13 genes (including TERT) and for 42 types of gene fusions in a series of 143 consecutive thyroid FNA samples with a cytologic diagnosis of follicular or Hurthle cell neoplasm or suspicious for follicular or Hurthle cell neoplasm. Molecular testing was retrospectively performed for 91 samples and prospectively performed for the remaining 52. Results for performance characteristics of the TERT variant alone were reported. Four of 39 total cancers were identified as TERT-positive (2 were unique diagnostic events); there were not TERT-positive results in the benign samples.

Liu and Xing (2014) described the performance of TERT as a single-gene test. FNA biopsy specimens were obtained preoperatively from thyroid nodules of 308 patients who underwent thyroidectomy. The percentage of samples that showed indeterminate cytologic findings on FNA biopsy was not described. The disposition of samples meeting eligibility criteria and a number of samples that did not produce results, were not described. Standard PCR was performed for direct genomic DNA sequencing to identify TERT promoter variants (C228T and C250T). One hundred twenty-nine (42%) of the samples were positive for thyroid cancer by pathology following surgery (111 PTC, 18 follicular thyroid carcinomas). TERT promoter variants C228T and C250T were found in 9 cases of thyroid cancer and no TERT variants were found in the 179 benign samples.

In 2016, Liu et. al. evaluated TERT promoter mutations in thyroid cancer which concluded, it has been less than three years since the initial report on TERT promoter mutations in thyroid cancer, while substantial progress has occurred in this exciting new field. Much has been known about the biological and clinical relevance of these mutations in thyroid cancer in this short time. Studies from various populations and regions in the world uniformly found TERT promoter mutations to be present in thyroid cancers, but not benign thyroid tumors, and be more common in aggressive types of thyroid cancers. These mutations are also more commonly associated with aggressiveness tumor behaviors and poor clinical outcomes, including tumor recurrence and patient mortality. A particular interesting and important aspect of TERT promoter mutations in papillary thyroid carcinoma (PTC) is their association with the BRAF V600E mutation

and the robust synergistic impact of the coexisting two mutations on aggressive clinicopathological outcomes of PTC, particularly tumor recurrence and patient mortality. These results are consistent with the proposed model in which TERT promoter mutations create consensus binding sites for ETS transcriptional factors for the later to activate the expression of TERT, a process that can be upregulated by the BRAF V600E/MAP kinase signaling pathway. A similar synergistic effect between TERT promoter mutations and RAS mutations, likely through activating the PI3K pathway, may also exist in thyroid cancer. These clinicopathological data strongly support a prominent role of TERT promoter mutations in the tumorigenesis and progression of thyroid cancer, which is well corroborated by previous results on similar differential expression patterns of TERT in benign and malignant thyroid tumors. As such, TERT promoter mutations are promising diagnostic and prognostic genetic makers for thyroid cancer, which, in combination with BRAF V600E mutation or other genetic markers (e.g., RAS mutations), are proving to be clinically useful for the management of thyroid cancer. Future studies will specifically define such clinical utilities, clarify the biological mechanisms, and explore the potential therapeutic targets of TERT promoter mutations in thyroid cancer.

Decaussin-Petrucci et. al. (2017) evaluated the feasibility and performance of molecular profiling in routine practice by testing LB-FNA (liquid based FNA) for BRAF, N/HRAS and TERT mutations on thyroid FNAs with indeterminate cytology. The study was a large prospective cohort of 326 cases, including 61 atypia of undetermined significance, 124 follicular neoplasms, 72 suspicious for malignancy and 69 malignant cases. Diagnosis of malignancy was confirmed by histology on paired surgical specimen. Mutated LB-FNAs were significantly associated with malignancy regardless of the cytological classification. Overall sensitivity was 60% and specificity 89%. Importantly, in atypia of undetermined significance and follicular neoplasm patients undergoing surgery according to the Bethesda guidelines, negative predictive values were 85.4% and 90% respectively. TERT promoter mutation was rare but very specific for malignancy (5.5%) suggesting that it could be of interest in patients with indeterminate cytology.

In summary, no studies of validity of the marketed version of TERT single-gene test were identified. Three studies reported information on TERT testing sufficient to calculate performance characteristics. The sample sizes of the included studies are approximately 150 to 350, with the prevalence of TERT variants between 3% and 7% and prevalence of cancer between 27% and 42%. Specificity was 100% in all studies (i.e., there were no false positives); however, the confidence intervals for PPV were extremely wide.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome 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 of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Testing for specific variants associated with thyroid cancer (e.g., BRAF V600E and RET variants, RET/PTC and PAX8/PPARy rearrangements) is generally designed to "rule in" cancer in nodules that have indeterminate cytology on FNA. A potential area for clinical utility for this type of variant testing would be in informing preoperative planning for thyroid surgery following initial thyroid FNA, such as planning for a hemi vs 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. 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 single nucleotide variants; RET/PTC1, RET/PTC3, and PAX8/PPARy rearrangements) was prospectively obtained for all FNAs with indeterminate cytology (follicular lesion of undetermined significance, follicular neoplasm, suspicious for malignancy), and for selective FNAs at the request of the managing physician for selected nodules with benign or nondiagnostic cytology. The study also included a second cohort of patients who did not have molecular testing results available. For patients treated with molecular diagnosis, a positive molecular diagnostic test was considered 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 managed with 349 without molecular diagnostics. Positive molecular testing results were obtained in 56 (17% of those managed with molecular diagnostics) patients, most commonly RAS variants (42/56 [75%]), followed by BRAF V600E (10/56 [18%]) and BRAF K601E (2/56 [4%]) variants, and PAX8/PPARy rearrangements (2/56 [4%]). Compared with those managed without molecular diagnostics (63%), patients managed with molecular diagnostics (69%) were nonsignificantly less likely to undergo total thyroidectomy as an initial procedure ($p=0.08$). However, they had nonsignificantly higher rates of central compartment lymph node dissection (21% vs 15%, $p=0.06$). Across both cohorts, 25% (170/671) of patients had clinically significant thyroid cancer, with no difference in thyroid cancer rates based on the type of initial surgery (26% for total thyroidectomy vs 22% for lobectomy, $p=0.3$). 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 a sensitivity and specificity for the diagnosis of thyroid cancer of 89% and 27%, retrospectively, with a

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) published a review of recommendations for the surgical management of FNA indeterminate nodules with various molecular genetic tests. This review reported on the estimated likelihood of malignancy in an FNA indeterminate nodule depending on the results of the Afirma GEC test (described above) and other molecular testing designed to rule in malignancy. Depending on the estimated pre-biopsy likelihood of malignancy, recommendations for surgery included observation, active surveillance, repeat FNA, diagnostic lobectomy or oncologic thyroidectomy.

No clinical utility studies for Afirma Xpression Atlas (XA) were identified.

Summary
The available evidence has suggested that use of variant 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 variant testing for genes associated with malignancy in thyroid cancer comes from a single-center retrospective study that reported surgical decisions and pathology findings in patients managed with and without molecular diagnostics. There is potential clinical utility for identifying malignancy with high certainty on FNA if such testing permits better pre-operative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. An American Thyroid Association (ATA) statement provides some guidelines for surgeons managing patients with indeterminate nodules. However, adoption of these guidelines in practice and outcomes associated with them are uncertain.

Based on review of the peer reviewed medical literature Afirma Xpression Atlas (XA) became commercially available to most of the United States in 2018. One retrospective analytical and clinical validation study was found. Given the lack of peer reviewed publications, no conclusions can be made regarding the clinical validity, clinical utility, or the overall value of the Xpression Atlas as an add-on to the Afirma GSC test. Further studies are needed to establish clinical utility. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Based on review of the peer reviewed medical literature the TERT (telomerase reverse transcriptase) promoter mutations may be performed as part of a mutation panel (ThyGenX) or on an individual basis, and TERT promoter mutations are commonly associated with aggressive tumor behaviors and poor clinical outcomes including tumor recurrence and patient mortality. While the studies may be promising as a diagnostic and prognostic genetic marker for thyroid cancer, future studies are needed to more

specifically define clinical utility, clarify the biological mechanisms of the role of TERT promoter mutations in thyroid cancer, and explore and establish therapeutic utilities of targeting TERT for thyroid cancer. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Molecular Markers to Rule-Out and Rule-In Malignancy

Clinical Context and Test Purpose
The purpose of the ThyroSeq v2 or the ThyroSeq v3 test and the combined ThyGenX/ThyGeNEXT Thyroid Oncogene Panel and ThyraMIR microRNA classifier in individuals with indeterminate findings on FNAs of thyroid nodules is to predict malignancy and inform surgical planning decisions with positive results using ThyroSeq v2 or the ThyGenX/ThyGeNEXT, and if negative, predict benignancy using ThyraMIR microRNA classifier to eliminate or necessitate the need for surgical biopsy and guide surgical planning.

The relevant population of interest includes individuals with indeterminate findings on FNAs of thyroid nodules. Patients with indeterminate findings presently proceed to surgical resection. The tests being considered are either the ThyroSeq test (ThyroSeq v3) or the combined use of ThyGenX/ThyGeNEXT Thyroid Oncogene Panel and ThyraMIR microRNA classifier testing. The potential beneficial outcome of primary interest is 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). Potentially 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 for thyroid cancer and delay in diagnosis.

ThyroSeq v2 and ThyroSeq v3 Test

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

A number of studies have evaluated whether testing for single nucleotide variants (SNVs) (either single variants or panels of variants) can be used to improve the sensitivity and specificity for diagnosing indeterminate FNA of the thyroid, with the goal of identifying mutations that predict malignancy in FNA samples.

In 2018, Steward et. al. studied the diagnostic accuracy of a multigene classifier (GC) test ThyroSeq v3 for cytologically indeterminate thyroid nodules in a prospective, blinded cohort study conducted at 10 medical centers with 782 patients with 1013 nodules enrolled. Eligibility criteria was met in 256 patients with 286 nodules, central pathology review was performed on 274 nodules. A total of 286 FNA samples from thyroid nodules underwent molecular analysis using the multigene classifier (GC) ThyroSeq v3. The

primary outcome was diagnostic accuracy of the test for thyroid nodules with Bethesda III and IV cytology. The secondary outcome was prediction of cancer by specific genetic alterations in Bethesda III to V nodules. Of the 286 cytologically indeterminate nodules, 206 (72%) were benign, 69 (24%) malignant, and 11 (4%) noninvasive follicular thyroid neoplasms with papillary-like nuclei (NIFTP). A total of 257 (90%) nodules (154 Bethesda III, 93 Bethesda IV, and 10 Bethesda V) had informative GC analysis, with 61%classified as negative and 39%as positive. In Bethesda III and IV nodules combined, the test demonstrated a 94% (95% CI, 86%-98%) sensitivity and 82% (95% CI, 75%-87%) specificity. With a cancer/NIFTP prevalence of 28%, the negative predictive value (NPV) was 97% (95% CI, 93%-99%) and the positive predictive value (PPV) was 66% (95% CI, 56%-75%). The observed 3% false-negative rate was similar to that of benign cytology, and the missed cancers were all low-risk tumors. Among nodules testing positive, specific groups of genetic alterations had cancer probabilities varying from 59% to 100%. The authors concluded, the study documents a high sensitivity and correspondingly high NPV of the ThyroSeq GC test for Bethesda III and IV indeterminate cytology nodules, which together with high specificity may prevent diagnostic surgeries in the majority of such patients. The availability of detailed genetic information in test-positive cases may help to further inform individualized treatment for these patients after integration with imaging and other clinical information.

Variants Association with Malignancy

Ferraz et. al. (2011) evaluated 20 publications that reported on the type and number of variants in cases of FNA of the thyroid diagnosed as indeterminate and compared with results with final histology after surgical resection. Sixteen studies analyzed 1 variant (e.g., BRAF variant or RET/PTC rearrangement) and 4 studies analyzed a panel of several variants (BRAF and RAS variants, RET/PTC and PAX8/PPARy rearrangements). The detection of a variant in a histologically (surgically resected) benign thyroid lesion was categorized as a false positive case, detecting no variant in an FNA sample from a histologically benign surgical sample was considered a true negative, and finding no variant in a histologically malignant lesion was categorized as a false negative. Based on 4 studies that examined a panel of variants, there was a broad sensitivity range (38%-85.7%; mean, 63.7%), a mean specificity of 98% (range, 95%-100%), mean false-positive rate of 1.25% (range, 0%-4%), and mean false-negative rate of 9% (range, 1%-21%). Based on 2 studies the examined RET/PTC rearrangement, mean sensitivity was 55% (range, 50%-60%), specificity 100%, a false-positive rate of 0% and mean false-negative rate 3.5% (91%-6%). Based on 3 studies that examined BRAF variants, mean sensitivity was 13% (range, 0%-37.5%), mean specificity was 92.3% (range, 75%-100%), mean false-positive rate was 0.5% (0%-1%), and mean false-negative rate was 6% (range, 3%-12%). Authors concluded that testing for a panel of variants leads to an improvement in the sensitivity and specificity for indeterminate FNA of the thyroid but that further standardizations and further molecular markers are needed before broad application of molecular FNA cytology for the diagnosis of thyroid nodules.

In 2015, Fnais et. al. conducted a systematic review and meta-analysis of studies reporting on the test accuracy of BRAF variant testing in the diagnosis of PTC. The

review 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%).

ThyroSeq Next Generation Sequencing (NGS) Panel
The largest body of literature on variant testing for prediction of malignancy in indeterminate thyroid nodules is related to the development a NGS panel (ThyroSeq) that includes BRAF, RAS, RET/PTC, or PAX8/PPARy. Studies that address these panels are described in more detail; studies that include subsets of these variants or additional variants are summarized in the following section.

Nikiforov et. al. (2009) prospectively tested a panel of variants (BRAF, RAS, RET/PTC, PAX8/PPARy) in 470 FNA samples of thyroid nodules from 328 consecutive patients. Variant status was correlated with cytology and either surgical pathology diagnosis or follow up (mean, 34 months). Forty patients were excluded for poor quality specimens or loss to follow up. Sixty-nine patients (with 86 thyroid FNA samples) underwent surgery soon after completion of the cytologic evaluation; preoperative cytologic diagnosis was; positive for malignancy in 22 samples, indeterminate (including atypical and suspicious for malignancy) in 52 samples, and negative for malignancy in 12 samples. By FNA, 32 variants were found (18 BRAF, 8 RAS, 5 RET/PTC, 1 PAX8/PPARy); after surgery, 31 (97%) variant positive nodules were diagnosed as malignant on pathologic examination, and 1 (3%) as benign tumor. Thirteen of the 32 variant positive FNA samples had a definitive cytologic diagnosis of malignancy, whereas the rest were either indeterminate or negative for malignancy.

Of the remaining 219 patients, 147 (229 FNAs) who did not undergo surgery were followed using serial ultrasound with no change in the nodule status (124 patients) or using repeated FNA with cytology negative for malignancy (23 patients) and no variant found in the FNA material. These nodules were considered negative for malignancy. The remaining 72 patients who were initially in the follow-up group underwent subsequent surgery. Combining all 3 groups, the specificity for malignancy was high (99.7%), but the sensitivity of the molecular test alone was not (62%).

Ohori et. al. (2010) performed mutation screenings in 117 FNA samples classified as a follicular lesion of indeterminate significance/atypia of indeterminate significance. BRAF, RAS, RET/PTC, or PAX8/PPARy variants were detected in 10% of this category. The screening demonstrated that the probability of having a malignancy in this cytology category together with a detection of one of the somatic variants investigated was 100%, whereas the probability of having a thyroid malignancy without a mutation detected was 7.6%.

In 2011, Nikiforov et. al. reported results of a prospective study that assessed the clinical validity of a panel of variants to predict the likelihood of malignancy in thyroid nodules found indeterminate on FNA. The authors included 1056 consecutive samples with

indeterminate cytology on FNA that underwent variant testing, with 967 of those adequate for molecular analysis (653 follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS); 247 follicular or Hurthle cell neoplasm or suspicious for follicular neoplasm; 67 suspicious for malignant cells). One hundred seventeen of the samples were included in the Ohori et. al. study described above and summarized in the table below. Eighty-seven BRAF, RAS, RET/PTC, or PAX8/PPARy variants were detected. At the time of analysis, 479 patients had undergone thyroidectomy for further evaluation, providing a histopathologic diagnosis for 513 samples. The presence of a variant had a low sensitivity for predicting malignant histology (63%, 57%, 68% for samples with follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS), follicular or Hurthle cell neoplasm/suspicious for follicular neoplasm, and suspicious for malignant cells on cytology, respectively), but a high specificity (99%, 97%, 96%, respectively). The negative predictive value (NPV) for the variant analysis results was 94%, 86% and 72% for samples with follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS), follicular or Hurthle cell neoplasm/suspicious for follicular neoplasm, and suspicious for malignant cells on cytology, respectively. The authors concluded that variant analysis might be useful in surgical planning, such as determining whether patients should undergo a thyroid lobectomy or a total thyroidectomy as a first surgery.

In a subsequent study, Nikiforov et. al. (2014) evaluated the accuracy of an NGS panel that included tests for single nucleotide variants in 13 genes and 42 types of gene fusions (ThyroSeq v2 NGS panel) in a series of 143 consecutive thyroid FNA samples with a cytologic diagnosis of follicular or Hurthle cell neoplasm/suspicious for follicular or Hurthle cell neoplasm. Molecular testing was retrospectively performed for 91 samples and prospectively performed for the remaining 52. The prevalence of cancer on histology was 27.5% and 26.9% in the retrospective and prospective cohorts. In the retrospective cohort, of the 25 malignant nodules, 22 were PTCs, and 3 were follicular thyroid carcinomas (FTCs). In the prospective cohort, of the 14 malignant nodules, 11 were PTCs and 3 were FTCs. The performance of the ThyroSeq in both cohorts is shown in the table below:

Performance of ThyroSeq Panel in Nikiforov et. al. (2014) and Nikiforov et. al. (2015)

	Nikiforov et. la. 2014		Nikiforov et. al. (2015)
Mutation Testing Outcomes	**Retrospective (n=91)**	**Prospective (n=52)**	**Overall (N=143)**	**Patients with Unknown Outcome (N=98)**
Negative	64 (2 cancer; 62 benign)	37 (2 cancer; 35 benign)		73 (2 cancer; 71 benign)
Positive	27	15 (12 cancer; 3 benign)		26 (20 cancer; 6 benign)

	(23 cancer; 5 benign)			
Sensitivity (95% CI)	92%	86%	90% (80% to 99%)	90.9% (78.8% to 100%)
Specificity (95% CI)	94%	92%	93% (88 to 98%)	92.1% (86.0% to 98.2%)
PPV (95% CI)	85%	80%	83% (72% to 95%)	76.9% (60.7% to 93.1%)
NPV (95% CI)	97%	95%	96% (92% to 95%)	97.2% (78.8% to 100%)

CI: confidence interval; NPV negative predictive value; PPV positive predictive value

The authors noted that, compared with the gene panel used in their 2011 study, the NGS panel was associated with marked increase in NPV, with a similar positive predictive value (PPV). In this case, the authors proposed that the panel could be used to both "rule in" and "rule out" invasive cancers.

The same group (Nikiforov et. al 2015) reported the performance of a subsequent generation ThyroSeq panel (ThyroSeq v2.1) with an expanded gene panel in a series of 465 thyroid FNA samples with a diagnosis of atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS). Molecular analysis was performed prospectively in all patients. Ninety patients (96 nodules) underwent thyroid surgery, based on either patient preference, the presence of another nodule with a diagnosis of suspicious for malignancy or malignant on FNA, or positive molecular testing. An additional 2 patients were considered to have a definitive nonsurgical diagnosis of primary hyperparathyroidism based on biochemical testing.

In addition to studies that describe the clinical validity of the genes that comprise the ThyroSeq panel, studies have reported on the diagnostic performance of individual variants and combinations of variants to predict malignancy in thyroid nodules that are indeterminate on FNA. The results that pertain to the use of mutation testing in indeterminate thyroid nodules are summarized in the table below:

Studies of Clinical Validity of Molecular Markers to Predict Malignancy in Indeterminate Thyroid FNA Samples

Study (year)	Population	Genes Tested	Insufficient or Inadequate for Analysis	Measures of Agreement				
				Sen	Spec	PPV	NPV	Acc
Moses et. al. (2010)	110 indeterminate thyroid nodules	BRAF, KRAS, NRAS, RET/PTC1, RET/PTC3, NTRK1	2	38	95	67	79	77

Ohori et. al. (2010)	100 patients with 117 follicular lesions of undetermined significance/atypia of undetermined significance	BRAF, NRAS, HRAS, KRAS, RET/PTC1, RET/PTC3, PAX8/PPARy	NR	60	100	100	92	93
Cantara et. al. (2010)	41 indeterminate **and** 54 suspicious thyroid nodules	BRAF,H-K NRAS, RET/PTC, TRK, PAX8/PPARy	53	86	97	86	97	95
				80	100	100	47	83
Xing et. al. (2004)	25 indeterminate dominate nodules	BRAF	NR	14	100	100	48	52
Jara et. al. (2015)	66 nodules suspicious for PTC	BRAF	NR	46	88	88	44	61
Rossi et. al. (2015)	140 indeterminate **or** suspicious for malignancy or malignant nodules	BRAF	NR	90	100	100	93	96
				50	100	100	69	77
				40	100	100	14	46
Beaudenon-Huibregtset et. al. (2014)	53 nodules with indeterminate/non-diagnostic FNA	BRAF, HRAS, KRAS, NRAS, PAX8/PPARy, RET/PTC1, RET/PTC3		48	89	81	64	

Sen: sensitivity; Spec: specificity; PPV: positive predictive value; NPV: negative predictive value; Acc: accuracy; FNA: fine needle aspiration; PTC: papillary thyroid carcinoma

Additional studies report on differences in variant frequency in malignant versus benign tumors, and report on the sensitivity and specificity of gene testing in unselected populations (i.e., all patients with nodules, rather than just those with indeterminate cytology). These studies are summarized next.

Mathur et. al. collected thyroid FNA samples, thyroid tissue, clinical and histopathology data, and tumor genotyping for BRAF V600E, NRAS, and KRAS variants, and RET/PTC1, RET/PTC3, and NTRK1 rearrangements for 341 patients with 423 dominant thyroid nodules. A cytologic examination of the samples showed that 51% were benign (25% were surgically resected), 21% were malignant, 11% were atypical lesions, 12% were follicular or Hurthle cell neoplasms, and 4% were suspicious for malignancy. On final analysis, 165 nodules were benign and 123 were malignant. Of the 423 FNA samples, 24 BRAF V600E, 7 KRAS, and 21 NRAS mutations, and 4 PAX8-PPARy, 3 RET/PTC1, and 2 RET/PTC3 rearrangements were detected. In all, 17 (10.3%) of 165 benign thyroid nodules had a variant compared with 26% (32/123) malignant tumors ($p<0.05$).

Eszlinger et. al. (2014) retrospectively analyzed a panel of variants (BRAF and RAS single nucleotide variants and PAX8/PPARy and RET/PTC rearrangements) in a sample of 310 thyroid air-dried FNA specimens with available corresponding FFPE thyroid biopsy samples (164 indeterminate, 57 malignant, and 89 benign on FNA). A total of 47 variants were detected on FNA: 22 BRAF, 13 NRAS, 3 HRAS variants, and 8 PAX8/PPARy and 1 RET/PTC rearrangements. The addition of variant analysis to cytology results was associated with a sensitivity of 75.3% and specificity of 90.4% for the detection of malignancy, with a PPV of 77.2% and NPV of 89.4%. The presence of BRAF mutation or a RET/PTC rearrangement was associated with cancer in 100% of samples.

The association between BRAF variants and PTC is supported by a report by Park et. al. (2015) on 294 patients with thyroid nodules whose FNA samples were evaluated with BRAF variants using 2 methods, real-time PCR with TaqMan minor groove-binding probes and allele-specific PCR using dual-priming oligonucleotides. The detection rate of PTC by BRAF variant testing by real time PCR and allele-specific PCR was 80.2% (95% CI, 71.9% to 86.9%) and 76.9% (95% CI, 68.3% to 84.0%), respectively.

In 2021, Li W et. al. evaluated the impact of ThyroSeq in the management of indeterminate thyroid nodules (ITN), including Bethesda III and IV nodules. ITNs that underwent ThyroSeq testing between 2016 and 2019 were retrospectively reviewed. A control cohort included ITNs without molecular testing. Cytological, molecular, and histological data were collected. We identified 202 ITNs that underwent molecular testing (128 in Bethesda III and 74 in Bethesda IV). Mutations were found in 58 nodules with mutation rates of 21.9% in Bethesda III and 40.5% in Bethesda IV. In this cohort, 49 cases had surgical resection with a resection rate of 24.3% (49/202, 15.6% in Bethesda III and 39.2% in Bethesda IV). Among the resected cases, 42 cases had positive molecular results. Thyroid cancer was diagnosed in 21 nodules with a malignancy detection rate of 10.4%. In the other cohort, we identified 236 ITNs (158 in Bethesda III and 78 in Bethesda IV). Surgical resection was performed in 127 cases, with a resection rate of 53.8% (127/236, 46.2% in Bethesda III and 69.2% in Bethesda IV). Thyroid cancer was diagnosed in 21 nodules, with a malignancy detection rate of 8.9%. The risk of malignancy (ROM) recalculated based on positive ThyroSeq results was significantly higher (21.4%-35.5% in Bethesda III and 50%-60% in Bethesda IV) than that without molecular testing (4.4%-9.6% in Bethesda III and 17.9%-25.9% in Bethesda IV). Authors concluded that ThyroSeq significantly decreased the surgical resection rate (from 53.8% to 24.3%) without significantly affecting the malignancy detection rate in ITNs. Furthermore, positive molecular testing significantly increased ROM in ITNs. We believe that the recalculated ROM should be incorporated into the management of ITNs.

Genetic Variants Association with Tumor Behavior

As reported in studies previously described the presence of BRAF mutations is strongly associated with malignancy in thyroid nodule FNA samples. BRAF variants have also

been associated with more aggressive clinicopathologic features in individuals who are diagnosed with PTC (papillary thyroid carcinoma).

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. The results of histopathologic follow-up correlated with 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 extrathyroidal extension or aggressive histologic features were positive for BRAF variant. The authors concluded that patients with an equivocal cytologic diagnosis and BRAF V600E mutation 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. A BRAF variant in preoperative FNA specimens was associated with poorer clinicopathologic outcomes for PTC. Compared with wild type allele, a BRAF mutation 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/recurrence was seen in 36% of BRAF variant-positive patients versus 12% of BRAF variant-negative patients, with an odds ratio 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/recurrence were 36% and 88% for all histologic subtypes of PTC. The authors concluded that preoperative BRAF variant testing of FNA specimens may 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.

ThyGenX/ThyGeNEXT Thyroid Oncogene Panel and ThyraMIR MicroRNA Classifier

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

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 atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) or follicular neoplasm (FN)/suspicious for a follicular neoplasm (SNF) across 12 endocrinology centers across the United States. Qualitative molecular results were compared with surgical histopathology to determine diagnostic performance and model clinical effect. Mutations were detected in 69% of nodules with malignant outcome. Among mutation negative specimens, miRNA testing correctly identified 64% of malignant cases and 98% of benign cases. The diagnostic sensitivity

and specificity of the combined algorithm was 89% (95% confidence interval [CI], 73-97%) and 85% (95% CI, 75-92%), respectively. At 32% cancer prevalence, 61% of the molecular results were benign with a negative predictive value of 94% (95% CI, 85-98%). Independently of variations in cancer prevalence, the test increased the yield of true benign results by 65% relative mRNA-based gene expression classification and decreased the rate of avoidable diagnostic surgeries by 69%. The authors concluded that a diagnostic algorithm combining miRNA expression and gene mutation detection yields clinically actionable molecular information in thyroid nodules with AUS/FLUS or FN/SFN cytology. Based on the high PPV and NPV of the MPT (multiplatform mutation test), it is reasonable to propose that patients with positive (malignant) MPT results may be sent to surgery while patients with negative (benign) MPT results may benefit from a more conservative management, i.e., active follow-up without surgery.

There is no published evidence evaluating the diagnostic accuracy or clinical utility of the combination test, ThyGeNEXT and ThyraMIR.

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 correctly therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct evidence for the 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 test and the combined ThyGenX/ThyGeNEXT and ThyraMIR diagnostic testing algorithm is lacking.

Chain of Evidence
A chain of evidence may be constructed to infer potential clinical utility of the combined diagnostic testing algorithm. Available evidence suggested that the use of variant testing using NGS in thyroid FNA samples is generally associated with a 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 a high enough NPV to identify which patients can undergo active surveillance over thyroid surgery. In the diagnostic algorithm that reflexes to the ThyraMIR after a negative ThyGenX/ThyGeNEXT result, patients receiving reflex testing could identify who may under active surveillance over thyroid surgery. A single study using a 17-variant panel with ThyraMIR showed a NPV of 94%. Therefore, the high NPV of ThyraMIR has the potential to accurately predict benignancy and triage patients to active surveillance.

Summary
The evidence for clinical validity and clinical utility of the combined ThyGenX and ThyraMIR is limited and consists of retrospective studies. There is no evidence of analytic validity, clinical validity or clinical utility of the newly named expanded test, ThyGeNEXT. There is no published evidence evaluating the diagnostic accuracy or clinical utility of the combination test, ThyGeNEXT and ThyraMIR. However, until these studies are performed, some organizations rely on expert opinion to guide the use of this test, which has yielded clinical input that supports the use of ThyraMIR and ThyGeNEXT in the following: FNA of thyroid nodules with indeterminate cytologic findings or Bethesda diagnostic category V (suspicious for malignancy) 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.

Summary of Evidence
For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular markers to rule out malignancy to avoid surgical biopsy, the evidence includes a prospective clinical validity study with the Afirma Gene Expression Classifier (GEC), and a chain of evidence to support clinical utility. In a multicenter validation study, the Afirma GEC was reported to have high negative predictive value (NPV; range 90%-95%). These results are supported by an earlier development and clinical validation study (Chudova et. al.), but the classifiers used in the two studies do not appear to be identical. In other multicenter and multiple single-center studies, there is suggestive evidence that rates of malignancy are low in Afirma benign patients, but the exact NPV is unknown. The available evidence suggests that physician decision making about surgery is altered by GEC results, although long term follow-up of patients with thyroid nodules who avoided surgery based on GEC results is limited. A chain of evidence can be constructed to establish the potential for clinical utility with GEC testing in cytologically indeterminate lesions, but with only a single study of the marketed test reporting a turn NPV, the clinical validity is uncertain. Based on clinical input received in 2017 from physician specialty societies and academic medical centers, the clinical input supports that this testing for certain indications provides a clinically meaningful improvement in net health outcome and are consistent with generally accepted medical practice (see below information on Clinical Input).

For the RosettaGX Reveal test, no prospective clinical studies were identified and there is no evidence directly demonstrating improved outcomes in patients managed with RosettaGX Reveal. The evidence is insufficient to determine the effects of technology on health outcomes. Based on clinical input received in 2017 from physician specialty societies and academic medical centers, the clinical input provided does not support this testing as this testing does not provide a clinically meaningful improvement in net health outcome or is consistent with generally accepted medical practice.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular markers to rule in malignancy to guide surgical planning, the evidence includes prospective and retrospective studies of clinical validity.

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 with an initial complete thyroidectomy. Prospective studies in additional populations are needed to validate these results. Variant analysis does not achieve enough NPV to identify which patients can undergo active surveillance over thyroid surgery. 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. The evidence is insufficient to determine the effects of the technology on health outcomes. However, based on clinical input received in 2017 from physician specialty societies and academic medical centers, the clinical input supports that this testing for certain indications provides a clinically meaningful improvement in net health outcome and are consistent with generally accepted medical practice (see below information on Clinical Input).

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular markers to rule out malignancy and to avoid surgical biopsy and rule into surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq v2 and two retrospective clinical validation studies that utilized a predicate test 17-variant panel (miRInform) test to the current ThyGenX and ThyraMIR. In a retrospective validation study on FNA samples, the 17-variant panel (miRInform) test and ThyraMIR had a sensitivity of 89% and a NPV of 94%. Pooled retrospective and prospective clinical validation studies of ThyroSeq v2 have reported a combined negative predictive value (NPV) of 96% and a positive predictive value (PPV) of 83%. 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 v2 test and the combined ThyGenX and ThyraMIR testing would rely on establishing clinical validity. The evidence is insufficient to determine the effects of the technology on health outcomes. However, based on clinical input received in 2017 from physician specialty societies and academic medical centers, the clinical input supports that this testing for certain indications provides a clinically meaningful improvement in net health outcome and are consistent with generally accepted medical practice (see below information on Clinical Input).

Based on review of the peer reviewed medical literature the TERT (telomerase reverse transcriptase) promoter mutations may be performed as part of a mutation panel (ThyGenX) or on an individual basis, and TERT promoter mutations are commonly associated with aggressive tumor behaviors and poor clinical outcomes including tumor recurrence and patient mortality. While the studies may be promising as a diagnostic and prognostic genetic marker for thyroid cancer, future studies are needed to more specifically define clinical utility, clarify the biological mechanisms of the role of TERT promoter mutations in thyroid cancer, and explore and establish therapeutic utilities of

targeting TERT for thyroid cancer. The evidence is insufficient to determine the effects of the technology on net health outcomes.

Clinical Input from Physician Specialty Societies and Academic Medical Centers
In 2017, clinical input was sought by Blue Cross Blue Shield Association (BCBSA) to help determine whether the evidence and clinical experience supports a clinical benefit of testing for molecular markers in fine needle aspirates (FNA) of the thyroid for management of individuals with thyroid nodule(s) with an indeterminate finding on the fine needle aspirate. 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 academic center and 7 physicians from 2 health systems. Based on the evidence and independent clinical input, the clinical input supports that the following indications provide a clinically meaningful improvement in net health outcome and are consistent with generally accepted medical practice.

Use of the following types of molecular marker testing in fine needle aspirate 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) to rule out malignancy and to avoid surgical biopsy:

- Afirma Gene Expression Classifier; or
- ThyroSeq v2

Use of the following type of molecular markers testing in FNA of thyroid nodules with indeterminate cytological findings or Bethesda diagnostic category V – suspicious for malignancy 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

Based on the evidence and independent clinical input, the clinical input does not support whether the following indications provides a clinically meaningful improvement in net health outcome or is consistent with generally accepted medical practice:

- Use of the following types of molecular marker testing in FNA of thyroid nodules:
    - RosettaGX Reveal

Practice Guidelines and Position Statements

American Thyroid Association (ATA)
In 2015, the American Thyroid Association (ATA) issued updated guidelines on the management of adult patients with thyroid nodules and differentiated thyroid cancer.

These guidelines make the following recommendations regarding molecular diagnostic testing:

- Thyroid nodule FNA cytology should be reported using diagnostic groups outlined in the Bethesda System for Reporting Thyroid Cytopathology. (Strong recommendation, Moderate-quality evidence)
- Indeterminate cytology (AUS/FLUS, FN, SUSP) – what are the principles of the molecular testing for FNA samples
    - If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results. (Strong recommendation, Low quality evidence)
    - If intended for clinical use, molecular testing should be performed in Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP) – certified molecular laboratories, or the international equivalent because reported quality assurance practices may be superior compared to other settings. (Strong recommendation, Low-quality evidence)
    - AUS/FLUS Cytology
        - For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision making. (Weak recommendation. Moderate-quality evidence)
        - 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 recommendation, Low quality evidence)
    - Follicular neoplasm/suspicious for follicular neoplasm cytology
        - 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 recommendation, Moderate quality evidence)
        - If molecular testing is either not performed or inconclusive, surgical excision may be considered for removal and definitive diagnosis of an FN/SFN thyroid nodule. (Strong recommendation, Low quality evidence)
    - Suspicious for malignancy cytology

- If the cytology is reported as suspicious for papillary carcinoma (SUSP), surgical management should be similar to that of malignant cytology, depending on clinical risk factors, sonographic features, patient preference, and possibly results of mutational testing (if performed). (Strong recommendation, Low quality evidence)
- After consideration of clinical and sonographic features, mutational testing for BRAF or the seven gene mutation marker panel (BRAF, RAS, RET/PTC, PAX8/PPARy) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision making. (Weak recommendation, Moderate quality evidence)

National Comprehensive Cancer Network (NCCN)
The National Comprehensive Cancer Network (NCCN) guideline thyroid carcinoma version 3.2021 make the following recommendations on the use of molecular diagnostics in thyroid cancer:

Molecular diagnostic testing to detect individual mutations (e.g., BRAF V600E, RET/PTC, RAS, PAX8/PPAR [peroxisome proliferator-activated receptors] gamma) or pattern recognition approaches using molecular classifiers may be useful in the evaluation of FNA samples that are indeterminate to assist in management decisions. The BRAF V600E mutation occurs in about 45% of patients with papillary carcinoma and is the most common mutation. Some studies have linked the BRAF 600E mutation to poor prognosis, especially when occurring with TERT promoter mutation. The choice of the precise molecular test depends on the cytology and the clinical question being asked. Indeterminate groups include: 1) follicular or Hurthle cell neoplasms (Bethesda IV); and 2) AUS/FLUS (Bethesda III). The NCCN Panel recommends consideration of molecular diagnostic testing for these indeterminate groups.

Molecular diagnostic testing may include multigene assays (e.g., the GEC) or individual mutation analysis. The GEC measures the expression of at least 140 genes. In addition to their utility in diagnostics, molecular markers are beneficial for making decisions about targeted therapy options for advanced disease and for informing eligibility for some clinical trials. In addition, the presence of some mutations may have prognostic importance.

A minority of panelists expressed concern regarding active nodule surveillance of follicular lesions because they were perceived as potentially pre-malignant lesions with a very low, but unknown, malignant potential if not surgically resected (leading to recommendations for either active surveillance or considering lobectomy in lesions classified as benign by molecular testing). Clinical risk factors, sonographic patterns, and patient preference can help determine whether active surveillance or lobectomy is appropriate for these patients. If molecular diagnostics are technically inadequate, then FNA may be repeated. Guidance regarding nodule surveillance from the ATA and the ACR TI-RADS should be followed.

Rather than proceeding to immediate surgical resection to obtain a definitive diagnosis for these indeterminate FNA cytology groups (follicular lesions), patients can be followed with active surveillance if the application of a specific molecular diagnostic test (in conjunction with clinical and ultrasound features) results in a predicted risk of malignancy that is comparable to the rate seen in cytologically benign thyroid FNAs (approximately $\leq$ 5%). It is important to note that the predictive value of molecular diagnostics may be significantly influenced by the pre-test probability of disease associated with the various FNA cytology groups. Furthermore, in the cytologically indeterminate groups, the risk of malignancy from FNA can vary widely between institutions. Because the published studies have focused primarily on adult patients with thyroid nodules, the diagnostic utility of molecular diagnostics in pediatric patients remains to be defined. Therefore, proper implementation of molecular diagnostics into clinical care requires an understanding of both the performance characteristics of the specific molecular tests and its clinical meaning across a range of pre-test disease probabilities.

When a diagnosis of thyroid carcinoma is promptly established using FNA the tumor is often confined to the thyroid or as metastasized only to regional nodes; thus, patients can be cured. However, as many as 5% of patients with papillary carcinoma and up to 10% of those patients with follicular or Hurthle cell carcinoma have tumors that aggressively invade structures in the neck or have produced distant metastases. Such cancers are difficult to cure.

American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE) and Associazone Medici Endocrinology (AME)
In 2016, the American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE) and Associazone Medici Endocrinology (AME) updated its joint guideline and made the following statements:

Patient specific characteristics, the prevalence of cancer within a given population, as well as the distribution and diagnostic accuracy for each cytologic classification have substantial impacts on assessing the odds of malignancy. This was highlighted in a 2012 meta-analysis showing that the malignancy rates across studies for AUS and FLUS ranged from 6 to 48% and 14 to 34%, respectively (154 [EL 2]). While molecular analysis of FNA genetic material from thyroid nodules shows great promise in refining the diagnosis, prognosis, and treatment of thyroid cancer, there are currently insufficient data to support a universal recommendation for molecular testing in the further categorization of “indeterminate” thyroid nodules.

Molecular testing for cytologically indeterminate thyroid nodules:

- Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the negative predictive values (NPVs) and positive predictive value (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 (GEC) for cytologically indeterminate nodules.

Role of molecular testing for deciding the extent of surgery:

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

How should patients with nodules that are negative at mutation testing be monitored

- Since the false-negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation negative nodules or nodules classified as benign by a GEC are still insufficient, close follow-up is recommended.

Regulatory Status

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

Commercially Available Molecular Diagnostics Tests for Indeterminate Thyroid Pathology

Test	Methodology	Analyte(s)	Report
Afirma GEC (e.g., Afirma Genomic Sequencing Classifier [GEC])	mRNA gene expression	167 genes	Benign/suspicious
Afirma BRAF	mRNA gene expression	1 gene	Negative/positive
Afirma Medullary Thyroid Carcinoma (MTC)	mRNA gene expression		Negative/positive
Afirma Xpression Atlas (XA)	mRNA gene expression	511 genes, 761 DNA variants, 130 fusion pairs	Specific gene variants
TERT single gene test	Unclear for commercially available test	1 gene	Specific gene variants

ThyroSeq v2	Next-generation sequencing	60+ genes	Specific gene variant/translocation
ThyroSeq v3	Next-generation sequencing	112 genes	Negative/positive
ThyGenX	Next-generation sequencing	8 genes	Specific gene variant/translocation
ThyGeNEXT	Next-generation sequencing	DNA Mutation Panel (150+ genes) and fusion pairs	Specific gene variant/translocation
ThyraMIR	microRNA expression	10 microRNAs	Negative/positive
RosettaGX Reveal	microRNA expression	24 microRNAs	Benign/suspicious for malignancy/high risk for medullary carcinoma

PRIOR APPROVAL

Not applicable.

POLICY

See related medical policy

- 02.01.20 Serum Tumor Markers in the Management of Malignancies

Afirma Gene Expression Classifier (GEC) (e.g., Afirma Genomic Sequencing Classifier [GSC])

Afirma gene expression classifier (GEC) (e.g., Afirma genomic sequencing classifier [GSC]) to assess fine needle aspirates (FNA) of thyroid nodules may be considered **medically necessary** when **ALL** of the following criteria are met:

- Thyroid nodule at least 1 cm on ultrasound; **and**
- Presence of indeterminate thyroid FNA cytopathology described as:
    - Atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (i.e., Bethesda category III); **OR**
    - Follicular neoplasm or suspicious for a follicular neoplasm (i.e., Bethesda category IV); **and**
- In whom surgical decision making would be affected by tests results.

Afirma gene expression classifier (GEC) (e.g., Afirma genomic sequencing classifier [GSC]) testing will be considered **not medically necessary**, including but not limited to the following:

- Evaluation of fine need aspirates (FNA) cytology with any of the following Bethesda cytologic categories (see table above under description):

    - Non-diagnostic or unsatisfactory (insufficient) samples (i.e., Bethesda category I)
    - Benign (i.e., Bethesda category II)
    - Suspicious for malignancy (i.e., Bethesda category V)
    - Malignant (i.e., Bethesda category VI); **OR**
- Evaluation of specimen other than fine needle aspirate (FNA) of thyroid nodules; **OR**
- Evaluation of thyroid nodule less than 1 cm; **OR**
- Evaluation of thyroid nodule with high suspicion of malignancy based on clinical or ultrasonographic features.

Afirma Malignancy Classifiers - Afirma Medullary Thyroid Cancer (MTC) and Afirma BRAF

Afirma Malignancy Classifiers (Afirma MTC) and/or Afirma BRAF may be considered **medically necessary** when the following criteria are met:

- Afirma Malignancy Classifiers, BRAF and MTC (Medullary Thyroid Cancer) are intended to guide surgical decisions when the Afirma Gene Expression Classifier (GEC) result suggests the patient should be considered for surgery:
    - The Afirma BRAF test (detects the BRAF V600E mutation), following Afirma Gene Expression Classifier (GEC) with a result that is suspicious.
    - The Afirma Medullary Thyroid Cancer (MTC), in conjunction with the Afirma Gene Expression Classifier (GEC) for indeterminate thyroid FNA cytopathology or following Afirma Gene Expression Classifier (GEC) with a result that is suspicious or malignant.

ThyroSeq v3, ThyraMIR microRNA and ThyGenX/ThyGeNEXT

The use of ThyroSeq v3, ThyraMIR microRNA and ThyGenX/ThyGeNext to assess fine needle aspirates (FNA) of thyroid nodules may be considered **medically necessary** when ALL of the following criteria are met:

- Presence of indeterminate thyroid FNA cytopathology described as:
    - Atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (i.e., Bethesda category III); **OR**
    - Follicular neoplasm or suspicious for a follicular neoplasm (i.e. Bethesda category IV); **OR**
    - Suspicious findings (i.e., Bethesda category V suspicious for malignancy); and
- Thyroid nodule(s) without a strong clinical or radiologic finding suggestive of malignancy; **and**
- In whom surgical decision making would be affected by tests results:
    - Guide surgical planning for initial resection (hemi vs a total thyroidectomy or performance of central neck dissection), rather than a two-stage surgical biopsy followed by definitive surgery.

The use of ThyroSeq v3, ThyraMIR microRNA and ThyGenX/ThyGeNEXT to assess fine needle aspirates (FNA) of thyroid nodules not meeting the criteria outlined above is considered **investigational** as there is insufficient evidence to support a conclusion concerning net health outcomes.

RosettaGX Reveal Test
The RosettaGX Reveal test to assess fine needle aspirates (FNA) of thyroid nodules is considered **investigational**.

Based on the review of the peer review medical literature no prospective clinical studies were identified and the evidence is insufficient in demonstrating improved outcomes in patients managed with RosettaGX reveal.

Gene Expression Classifiers (GEC)
Gene expression classifiers (GEC), genetic variant analysis and molecular marker testing in fine needle aspirates of the thyroid, including but not limited to the following are considered **investigational** as there is insufficient evidence to support a conclusion concerning net health outcomes:

- TERT (telomerase reverse transcriptase) promoter mutations
- Afirma Xpression Atlas (XA)

PROCEDURE CODES AND BILLING GUIDELINES

To report provider services, use appropriate CPT* codes, Alpha Numeric (HCPCS level 2) codes, Revenue codes, and/or ICD diagnosis codes.

- 81210 BRAF, gene analysis V600 variant (used for Afirma malignancy classifier BRAF V600E testing)
- 81345 TERT (telomerase reverse transcriptase (e.g., thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (e.g., promoter region)
- 81406 Molecular Pathology Procedure Level 7 (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons) ACADVL (acyl-CoA dehydrogenase, very long chain) (e.g., very long chain acyl-coenzyme A dehydrogenase deficiency), full gene sequence ACTN4 (actinin, alpha 4) (e.g., focal segmental glomerulosclerosis), full gene sequence AFG3L2 (AFG3 ATPase family gene 3-like 2 [S. cerevisiae]) (e.g., spinocerebellar ataxia), full gene sequence AIRE (autoimmune regulator) (e.g., autoimmune polyendocrinopathy syndrome type 1), full gene sequence ALDH7A1 (aldehyde dehydrogenase 7 family, member A1) (e.g., pyridoxine-dependent epilepsy), full gene sequence ANO5 (anoctamin 5) (e.g., limb-girdle muscular dystrophy), full gene sequence ANOS1 (anosmin-1) (e.g., Kallmann syndrome 1), full gene sequence APP (amyloid beta [A4] precursor protein) (e.g., Alzheimer disease), full gene sequence ASS1 (argininosuccinate synthase 1) (e.g., citrullinemia type I), full

gene sequence ATL1 (atlastin GTPase 1) (e.g., spastic paraplegia), full gene sequence ATP1A2 (ATPase, Na+/K+ transporting, alpha 2 polypeptide) (e.g., familial hemiplegic migraine), full gene sequence ATP7B (ATPase, Cu++ transporting, beta polypeptide) (eg, Wilson disease), full gene sequence BBS1 (Bardet-Biedl syndrome 1) (e.g., Bardet-Biedl syndrome), full gene sequence BBS2 (Bardet-Biedl syndrome 2) (e.g., Bardet-Biedl syndrome), full gene sequence BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (e.g., maple syrup urine disease, type 1B), full gene sequence BEST1 (bestrophin 1) (eg, vitelliform macular dystrophy), full gene sequence BMPR2 (bone morphogenetic protein receptor, type II [serine/threonine kinase]) (e.g., heritable pulmonary arterial hypertension), full gene sequence BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, Noonan syndrome), full gene sequence BSCL2 (Berardinelli-Seip congenital lipodystrophy 2 [seipin]) (eg, Berardinelli-Seip congenital lipodystrophy), full gene sequence BTK (Bruton agammaglobulinemia tyrosine kinase) (e.g., X-linked agammaglobulinemia), full gene sequence CACNB2 (calcium channel, voltage-dependent, beta 2 subunit) (eg, Brugada syndrome), full gene sequence CAPN3 (calpain 3) (e.g., limb-girdle muscular dystrophy [LGMD] type 2A, calpainopathy), full gene sequence CBS (cystathionine-beta-synthase) (eg, homocystinuria, cystathionine beta-synthase deficiency), full gene sequence CDH1 (cadherin 1, type 1, E-cadherin [epithelial]) (eg, hereditary diffuse gastric cancer), full gene sequence CDKL5 (cyclin-dependent kinase-like 5) (e.g., early infantile epileptic encephalopathy), full gene sequence CLCN1 (chloride channel 1, skeletal muscle) (e.g., myotonia congenita), full gene sequence CLCNKB (chloride channel, voltage-sensitive Kb) (e.g., Bartter syndrome 3 and 4b), full gene sequence CNTNAP2 (contactin-associated protein-like 2) (e.g., Pitt-Hopkins-like syndrome 1), full gene sequence COL6A2 (collagen, type VI, alpha 2) (e.g., collagen type VI-related disorders), duplication/deletion analysis CPT1A (carnitine palmitoyltransferase 1A [liver]) (e.g., carnitine palmitoyltransferase 1A [CPT1A] Molecular pathology procedure, Level 7 (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons) ACADVL (acyl-CoA dehydrogenase, very long chain) (e.g., very long chain acyl-coenzyme A dehydrogenase deficiency), full gene sequence ACTN4 (actinin, alpha 4) (e.g., focal segmental glomerulosclerosis), full gene sequence AFG3L2 (AFG3 ATPase family gene 3-like 2 [S. cerevisiae]) (e.g., spinocerebellar ataxia), full gene sequence AIRE (autoimmune regulator) (e.g., autoimmune polyendocrinopathy syndrome type 1), full gene sequence ALDH7A1 (aldehyde dehydrogenase 7 family, member A1) (e.g., pyridoxine-dependent epilepsy), full gene sequence ANO5 (anoctamin 5) (e.g., limb-girdle muscular dystrophy), full gene sequence ANOS1 (anosmin-1) (e.g., Kallmann syndrome 1), full gene sequence APP (amyloid beta [A4] precursor protein) (e.g., Alzheimer disease), full gene sequence ASS1 (argininosuccinate synthase 1) (e.g., citrullinemia type I), full gene sequence ATL1 (atlastin GTPase 1) (e.g., spastic paraplegia), full gene sequence ATP1A2 (ATPase, Na+/K+ transporting, alpha 2 polypeptide) (e.g., familial hemiplegic migraine), full gene sequence ATP7B (ATPase, Cu++ transporting, beta polypeptide) (e.g., Wilson

disease), full gene sequence BBS1 (Bardet-Biedl syndrome 1) (e.g., Bardet-Biedl syndrome), full gene sequence BBS2 (Bardet-Biedl syndrome 2) (e.g., Bardet-Biedl syndrome), full gene sequence BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (e.g., maple syrup urine disease, type 1B), full gene sequence BEST1 (bestrophin 1) (e.g., vitelliform macular dystrophy), full gene sequence BMPR2 (bone morphogenetic protein receptor, type II [serine/threonine kinase]) (e.g., heritable pulmonary arterial hypertension), full gene sequence BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, Noonan syndrome), full gene sequence BSCL2 (Berardinelli-Seip congenital lipodystrophy 2 [seipin]) (e.g., Berardinelli-Seip congenital lipodystrophy), full gene sequence BTK (Bruton agammaglobulinemia tyrosine kinase) (e.g., X-linked agammaglobulinemia), full gene sequence CACNB2 (calcium channel, voltage-dependent, beta 2 subunit) (e.g., Brugada syndrome), full gene sequence CAPN3 (calpain 3) (e.g., limb-girdle muscular dystrophy [LGMD] type 2A, calpainopathy), full gene sequence CBS (cystathionine-beta-synthase) (e.g., homocystinuria, cystathionine beta-synthase deficiency), full gene sequence CDH1 (cadherin 1, type 1, E-cadherin [epithelial]) (e.g., hereditary diffuse gastric cancer), full gene sequence CDKL5 (cyclin-dependent kinase-like 5) (e.g., early infantile epileptic encephalopathy), full gene sequence CLCN1 (chloride channel 1, skeletal muscle) (e.g., myotonia congenita), full gene sequence CLCNKB (chloride channel, voltage-sensitive Kb) (e.g., Bartter syndrome 3 and 4b), full gene sequence CNTNAP2 (contactin-associated protein-like 2) (e.g., Pitt-Hopkins-like syndrome 1), full gene sequence COL6A2 (collagen, type VI, alpha 2) (e.g., collagen type VI-related disorders), duplication/deletion analysis CPT1A (carnitine palmitoyltransferase 1A [liver]) (e.g., carnitine palmitoyltransferase 1A [CPT1A] deficiency), full gene sequence CRB1 (crumbs homolog 1 [Drosophila]) (e.g., Leber congenital amaurosis), full gene sequence CREBBP (CREB binding protein) (e.g., Rubinstein-Taybi syndrome), duplication/deletion analysis DBT (dihydrolipoamide branched chain transacylase E2) (e.g., maple syrup urine disease, type 2), full gene sequence DLAT (dihydrolipoamide S-acetyltransferase) (e.g., pyruvate dehydrogenase E2 deficiency), full gene sequence DLD (dihydrolipoamide dehydrogenase) (e.g., maple syrup urine disease, type III), full gene sequence DSC2 (desmocollin) (e.g., arrhythmogenic right ventricular dysplasia/cardiomyopathy 11), full gene sequence DSG2 (desmoglein 2) (e.g., arrhythmogenic right ventricular dysplasia/cardiomyopathy 10), full gene sequence DSP (desmoplakin) (e.g., arrhythmogenic right ventricular dysplasia/cardiomyopathy 8), full gene sequence EFHC1 (EF-hand domain [C-terminal] containing 1) (e.g., juvenile myoclonic epilepsy), full gene sequence EIF2B3 (eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa) (e.g., leukoencephalopathy with vanishing white matter), full gene sequence EIF2B4 (eukaryotic translation initiation factor 2B, subunit 4 delta, 67kDa) (e.g., leukoencephalopathy with vanishing white matter), full gene sequence EIF2B5 (eukaryotic translation initiation factor 2B, subunit 5 epsilon, 82kDa) (e.g., childhood ataxia with central nervous system hypomyelination/vanishing white matter), full gene sequence ENG (endoglin) (e.g., hereditary hemorrhagic

telangiectasia, type 1), full gene sequence EYA1 (eyes absent homolog 1 [Drosophila]) (eg, branchio-oto-renal [BOR] spectrum disorders), full gene sequence F8 (coagulation factor VIII) (e.g., hemophilia A), duplication/deletion analysis FAH (fumarylacetoacetate hydrolase [fumarylacetoacetase]) (e.g., tyrosinemia, type 1), full gene sequence FASTKD2 (FAST kinase domains 2) (e.g., mitochondrial respiratory chain complex IV deficiency), full gene sequence FIG4 (FIG4 homolog, SAC1 lipid phosphatase domain containing [S. cerevisiae]) (eg, Charcot-Marie-Tooth disease), full gene sequence FTSJ1 (FtsJ RNA methyltransferase homolog 1 [E. coli]) (e.g., X-linked mental retardation 9), full gene sequence FUS (fused in sarcoma) (e.g., amyotrophic lateral sclerosis), full gene sequence GAA (glucosidase, alpha; acid) (e.g., glycogen storage disease type II [Pompe disease]), full gene sequence GALC (galactosylceramidase) (e.g., Krabbe disease), full gene sequence GALT (galactose-1-phosphate uridylyltransferase) (e.g., galactosemia), full gene sequence GARS (glycyl-tRNA synthetase) (e.g., Charcot-Marie-Tooth disease), full gene sequence GCDH (glutaryl-CoA dehydrogenase) (e.g., glutaricacidemia type 1), full gene sequence GCK (glucokinase [hexokinase 4]) (e.g., maturity-onset diabetes of the young [MODY]), full gene sequence GLUD1 (glutamate dehydrogenase 1) (e.g., familial hyperinsulinism), full gene sequence GNE (glucosamine [UDP-N-acetyl]-2-epimerase/N-acetylmannosamine kinase) (e.g., inclusion body myopathy 2 [IBM2], Nonaka myopathy), full gene sequence GRN (granulin) (e.g., frontotemporal dementia), full gene sequence HADHA (hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase [trifunctional protein] alpha subunit) (e.g., long chain acyl-coenzyme A dehydrogenase deficiency), full gene sequence HADHB (hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase [trifunctional protein], beta subunit) (eg, trifunctional protein deficiency), full gene sequence HEXA (hexosaminidase A, alpha polypeptide) (e.g., Tay-Sachs disease), full gene sequence HLCS (HLCS holocarboxylase synthetase) (eg, holocarboxylase synthetase deficiency), full gene sequence HMBS (hydroxymethylbilane synthase) (e.g., acute intermittent porphyria), full gene sequence HNF4A (hepatocyte nuclear factor 4, alpha) (e.g., maturity-onset diabetes of the young [MODY]), full gene sequence IDUA (iduronidase, alpha-L-) (e.g., mucopolysaccharidosis type I), full gene sequence INF2 (inverted formin, FH2 and WH2 domain containing) (e.g., focal segmental glomerulosclerosis), full gene sequence IVD (isovaleryl-CoA dehydrogenase) (eg, isovaleric acidemia), full gene sequence JAG1 (jagged 1) (e.g., Alagille syndrome), duplication/deletion analysis JUP (junction plakoglobin) (e.g., arrhythmogenic right ventricular dysplasia/cardiomyopathy 11), full gene sequence KCNH2 (potassium voltage-gated channel, subfamily H [eag-related], member 2) (e.g., short QT syndrome, long QT syndrome), full gene sequence KCNQ1 (potassium voltage-gated channel, KQT-like subfamily, member 1) (e.g., short QT syndrome, long QT syndrome), full gene sequence KCNQ2 (potassium voltage-gated channel, KQT-like subfamily, member 2) (e.g., epileptic encephalopathy), full gene sequence LDB3 (LIM domain binding 3) (e.g., familial dilated cardiomyopathy, myofibrillar myopathy), full gene sequence LDLR (low density lipoprotein

receptor) (e.g. familial hypercholesterolemia) full gene sequence, LEPR (leptin receptor(e.g., obesity with hypogonadism), full gene sequence, LHCGR (luteinizing hormone/choriogonadotropin receptor) (eg, precocious male puberty), full gene sequence, LMNA (lamin A/C) (e.g., Emery-Dreifuss muscular dystrophy [EDMD1, 2 and 3] limb-girdle muscular dystrophy [LGMD] type 1B, dilated cardiomyopathy [CMD1A], familial partial lipodystrophy [FPLD2]), full gene sequence, LRP5 (low density lipoprotein receptor-related protein 5) (eg, osteopetrosis), full gene sequence, MAP2K1 (mitogen-activated protein kinase 1) (e.g., cardiofaciocutaneous syndrome), full gene sequence, MAP2K2 (mitogen-activated protein kinase 2) (e.g., cardiofaciocutaneous syndrome), full gene sequence, MAPT (microtubule-associated protein tau) (e.g., frontotemporal dementia), full gene sequence, MCCC1 (methylcrotonoyl-CoA carboxylase 1 [alpha]) (eg, 3-methylcrotonyl-CoA carboxylase deficiency), full gene sequence, MCCC2 (methylcrotonoyl-CoA carboxylase 2 [beta]) (eg, 3-methylcrotonyl carboxylase deficiency), full gene sequence, MFN2 (mitofusin 2) (e.g., Charcot-Marie-Tooth disease), full gene sequence, MTM1 (myotubularin 1) (e.g., X-linked centronuclear myopathy), full gene sequence, MUT (methylmalonyl CoA mutase) (e.g., methylmalonic acidemia), full gene sequence, MUTYH (mutY homolog [E. coli]) (e.g., MYH-associated polyposis), full gene sequence, NDUFS1 (NADH dehydrogenase [ubiquinone] Fe-S protein 1, 75kDa [NADH-coenzyme Q reductase]) (e.g., Leigh syndrome, mitochondrial complex I deficiency), full gene sequence, NF2 (neurofibromin 2 [merlin]) (eg, neurofibromatosis, type 2), full gene sequence, NOTCH3 (notch 3) (e.g., cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL]), targeted sequence analysis (eg, exons 1-23), NPC1 (Niemann-Pick disease, type C1) (e.g., Niemann-Pick disease), full gene sequence, NPHP1 (nephronophthisis 1 [juvenile]) (e.g., Joubert syndrome), full gene sequence, NSD1 (nuclear receptor binding SET domain protein 1) (e.g., Sotos syndrome), full gene sequence, OPA1 (optic atrophy 1) (e.g., optic atrophy), duplication/deletion analysis, OPTN (optineurin) (e.g., amyotrophic lateral sclerosis), full gene sequence, PAFAH1B1 (platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 [45kDa]) (eg, lissencephaly, Miller-Dieker syndrome), full gene sequence, PAH (phenylalanine hydroxylase) (e.g., phenylketonuria), full gene sequence, PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer), full gene sequence, PARK2 (Parkinson protein 2, E3 ubiquitin protein ligase [parkin]) (e.g., Parkinson disease), full gene sequence, *PAX2 (paired box 2)* (eg, renal coloboma syndrome), full gene sequence, *PC (pyruvate carboxylase)* (e.g., pyruvate carboxylase deficiency), full gene sequence, PCCA (propionyl CoA carboxylase, alpha polypeptide) (e.g., propionic acidemia, type 1), full gene sequence, PCCB (propionyl CoA carboxylase, beta polypeptide) (eg, propionic acidemia), full gene sequence, PCDH15 (protocadherin-related 15) (e.g., Usher syndrome type 1F), duplication/deletion analysis, PCSK9 (proprotein convertase subtilisin/kexin type 9) (e.g. familial hypercholesterolemia), full gene sequence, PDHA1 (pyruvate dehydrogenase [lipoamide] alpha 1) (eg, lactic acidosis), full gene sequence, PDHX (pyruvate dehydrogenase complex, component X) (e.g., lactic acidosis), full gene sequence,

PHEX (phosphate-regulating endopeptidase homolog, X-linked) (e.g., hypophosphatemic rickets), full gene sequence, PKD2 (polycystic kidney disease 2 [autosomal dominant]) (e.g., polycystic kidney disease), full gene sequence, PKP2 (plakophilin 2) (e.g., arrhythmogenic right ventricular dysplasia/cardiomyopathy 9), full gene sequence, PNKD (eg, paroxysmal nonkinesigenic dyskinesia), full gene sequence, POLG (polymerase [DNA directed], gamma) (e.g., Alpers-Huttenlocher syndrome, autosomal dominant progressive external ophthalmoplegia), full gene sequence, POMGNT1 (protein O-linked mannose beta1, 2-N acetylglucosaminyltransferase) (e.g., muscle-eye-brain disease, Walker-Warburg syndrome), full gene sequence, POMT1 (protein-O-mannosyltransferase 1) (e.g., limb-girdle muscular dystrophy [LGMD] type 2K, Walker-Warburg syndrome), full gene sequence, POMT2 (protein-O-mannosyltransferase 2) (e.g., limb-girdle muscular dystrophy [LGMD] type 2N, Walker-Warburg syndrome), full gene sequence, PPOX (protoporphyrinogen oxidase) (e.g., variegate porphyria), full gene sequence, PRKAG2 (protein kinase, AMP-activated, gamma 2 non-catalytic subunit) (e.g., familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome, lethal congenital glycogen storage disease of heart), full gene sequence, PRKCG (protein kinase C, gamma) (e.g., spinocerebellar ataxia), full gene sequence, PSEN2 (presenilin 2[Alzheimer's disease 4]) (e.g., Alzheimer's disease), full gene sequence, PTPN11 (protein tyrosine phosphatase, non-receptor type 11) (e.g., Noonan syndrome, LEOPARD syndrome), full gene sequence, PYGM (phosphorylase, glycogen, muscle) (e.g., glycogen storage disease type V, McArdle disease), full gene sequence, RAF1 (v-raf-1 murine leukemia viral oncogene homolog 1) (e.g., LEOPARD syndrome), full gene sequence, RET (ret proto-oncogene) (e.g., Hirschsprung disease), full gene sequence, RPE65 (retinal pigment epithelium-specific protein 65kDa) (e.g., retinitis pigmentosa, Leber congenital amaurosis), full gene sequence, RYR1 (ryanodine receptor 1, skeletal) (e.g., malignant hyperthermia), targeted sequence analysis of exons with functionally-confirmed mutations, SCN4A (sodium channel, voltage-gated, type IV, alpha subunit) (e.g., hyperkalemic periodic paralysis), full gene sequence, SCNN1A (sodium channel, nonvoltage-gated 1 alpha) (e.g., pseudohypoaldosteronism), full gene sequence, SCNN1B (sodium channel, nonvoltage-gated 1, beta) (e.g., Liddle syndrome, pseudohypoaldosteronism), full gene sequence, SCNN1G (sodium channel, nonvoltage-gated 1, gamma) (e.g., Liddle syndrome, pseudohypoaldosteronism), full gene sequence, SDHA (succinate dehydrogenase complex, subunit A, flavoprotein [Fp]) (e.g., Leigh syndrome, mitochondrial complex II deficiency), full gene sequence, SETX (senataxin) (e.g., ataxia), full gene sequence, SGCE (sarcoglycan, epsilon) (e.g., myoclonic dystonia), full gene sequence, SH3TC2 (SH3 domain and tetratricopeptide repeats 2) (e.g., Charcot-Marie-Tooth disease), full gene sequence, SLC9A6 (solute carrier family 9 [sodium/hydrogen exchanger], member 6) (e.g., Christianson syndrome), full gene sequence, SLC26A4 (solute carrier family 26, member 4) (e.g., Pendred syndrome), full gene sequence, SLC37A4 (solute carrier family 37 [glucose-6-phosphate transporter], member 4) (e.g., glycogen storage disease type Ib), full gene sequence, SMAD4 (SMAD

family member 4) (eg, hemorrhagic telangiectasia syndrome, juvenile polyposis), full gene sequence, SOS1 (son of sevenless homolog 1) (e.g., Noonan syndrome, gingival fibromatosis), full gene sequence, SPAST (spastin) (e.g., spastic paraplegia), full gene sequence, SPG7 (spastic paraplegia 7 [pure and complicated autosomal recessive]) (eg, spastic paraplegia), full gene sequence, STXBP1 (syntaxin-binding protein 1) (e.g., epileptic encephalopathy), full gene sequence, TAZ (tafazzin) (e.g., methylglutaconic aciduria type 2, Barth syndrome), full gene sequence, TCF4 (transcription factor 4) (eg, Pitt-Hopkins syndrome), full gene sequence, TH (tyrosine hydroxylase) (eg, Segawa syndrome), full gene sequence, TMEM43 (transmembrane protein 43) (e.g., arrhythmogenic right ventricular cardiomyopathy), full gene sequence, TNNT2 (troponin T, type 2 [cardiac]) (e.g., familial hypertrophic cardiomyopathy), full gene sequence, TRPC6 (transient receptor potential cation channel, subfamily C, member 6) (e.g., focal segmental glomerulosclerosis), full gene sequence, TSC1 (tuberous sclerosis 1) (e.g., tuberous sclerosis), full gene sequence, TSC2 (tuberous sclerosis 2) (e.g., tuberous sclerosis), duplication/deletion analysis, UBE3A (ubiquitin protein ligase E3A) (e.g., Angelman syndrome) full gene sequence, UMOD (uromodulin) (e.g., glomerulocystic kidney disease with hyperuricemia and isosthenuria), full gene sequence, VWF (von Willebrand factor) (von Willebrand disease type 2A), extended targeted sequence analysis (e.g., exons 11-16, 24-26, 51, 52), WAS (Wiskott-Aldrich syndrome [eczema-thrombocytopenia]) (e.g., Wiskott-Aldrich syndrome), full gene sequence

- 81479 Unlisted molecular pathology procedure (when specified as testing for thyroid molecular markers (may be used for the following tests: ThyGenX/ThyGeNEXT; RosettaGX Reveal)
- 81546 Oncology (thyroid), mRNA, gene expression analysis, of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (e.g., benign, or suspicious) (Afirma Gene Expression Classifier [GEC])
- 0018U Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences utilizing fine needle aspirate algorithm reported as a positive or negative result for moderate to high risk of malignancy (ThyraMIR)
- 0026U Oncology (thyroid), DNA and mRNA of 112 genes, next generation sequencing, fine needle aspirate of thyroid nodule algorithmic analysis reported as a categorical result (positive, high probability of malignancy or negative, low probability of malignancy) (ThyroSeq v3 Genomic Classifier)
- 0204U Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected (Afirma Xpression Atlas)
- 0287U Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high) (ThyroSeq CRC, CBLPath, Inc, University of Pittsburg Medical Center)

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POLICY HISTORY		
Date	Reason	Action
January 2022	Annual Review	Policy Renewed
January 2021	Annual Review	Policy Revised
January 2020	Annual Review	Policy Revised
January 2019	Annual Review	Policy Revised
January 2018	Annual Review	Policy Revised
January 2017		New Policy

New information or technology that would be relevant for Wellmark to consider when this policy is next reviewed may be submitted to:

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