



# Louisiana

## Molecular Markers in Fine Needle Aspirates of the Thyroid

**Policy #** 00332

Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

*Applies to all products administered or underwritten by Blue Cross and Blue Shield of Louisiana and its subsidiary, HMO Louisiana, Inc. (collectively referred to as the "Company"), unless otherwise provided in the applicable contract. Medical technology is constantly evolving, and we reserve the right to review and update Medical Policy periodically.*

### When Services May Be Eligible for Coverage

*Coverage for eligible medical treatments or procedures, drugs, devices or biological products may be provided only if:*

- *Benefits are available in the member's contract/certificate, and*
- *Medical necessity criteria and guidelines are met.*

Based on review of available data, the Company may consider the use of either Afirma Gene Expression Classifier or ThyroSeq v2 in fine needle aspirates of thyroid nodules with indeterminate cytologic findings (ie, Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) in patients who have the following characteristics to be **eligible for coverage**:

- Thyroid nodules without strong clinical or radiologic findings suggestive of malignancy.
- In whom surgical decision making would be affected by test results.

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

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

### When Services Are Considered Investigational

*Coverage is not available for investigational medical treatments or procedures, drugs, devices or biological products.*

Based on review of available data, the Company considers gene expression classifiers, genetic variant analysis, and molecular marker testing in fine needle aspirates of the thyroid not meeting criteria outlined above, including but not limited to use of RosettaGX Reveal, to be **investigational**.\*

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### **Policy Guidelines**

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

Use of molecular marker testing based on fine needle aspirate of a thyroid nodule to rule in malignancy prior to surgical biopsy may guide surgical planning, particularly factors such as choice of surgical facility provider to ensure that the capability is available to conduct a frozen section pathologic reading during surgical biopsy so that surgical approach may be adjusted accordingly in 1 surgery.

### **GENETICS NOMENCLATURE UPDATE**

Human Genome Variation Society (HGVS) nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). HGVS nomenclature is recommended by HGVS, the Human Variome Project, and the HUMAN Genome Organization.

The American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) standards and guidelines for interpretation of sequence variants represent expert opinion from ACMG, AMP, and the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

**Table PG1. Nomenclature to Report on Variants Found in DNA**

Previous	Updated	Definition
Mutation	Disease-associated variant	Disease-associated change in the DNA sequence
	Variant	Change in the DNA sequence
	Familial variant	Disease-associated variant identified in a proband for use in subsequent targeted genetic testing in first-degree relatives

**Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification**

Variant Classification	Definition
Pathogenic	Disease-causing change in the DNA sequence
Likely pathogenic	Likely disease-causing change in the DNA sequence
Variant of uncertain significance	Change in DNA sequence with uncertain effects on disease
Likely benign	Likely benign change in the DNA sequence
Benign	Benign change in the DNA sequence

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

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### **GENETIC COUNSELING**

Genetic counseling is primarily aimed at patients who are at risk for inherited disorders, and experts recommend formal genetic counseling in most cases when genetic testing for an inherited condition is considered. The interpretation of the results of genetic tests and the understanding of risk factors can be very difficult and complex. Therefore, genetic counseling will assist individuals in understanding the possible benefits and harms of genetic testing, including the possible impact of the information on the individual's family. Genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing. Genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

### **Background/Overview**

#### **THYROID NODULES**

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

#### **Diagnosis**

Sampling thyroid cells by fine needle aspirate (FNA) is currently the most accurate procedure to distinguish benign thyroid lesions 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, usually due to overlapping cytologic features between benign and malignant nodules; these nodules usually require surgery for a final diagnosis. Thyroid FNA cytology is classified by Bethesda System criteria into the following groups: nondiagnostic; benign; follicular lesion of undetermined significance (FLUS) or atypia of undetermined significance (AUS); follicular neoplasm (or suspicious for follicular neoplasm); suspicious for malignancy; and malignant. Lesions with FNA cytology in the AUS or FLUS or follicular neoplasm categories are often considered indeterminate.

#### **Management**

There is some individualization of management for patients with FNA-indeterminate nodules, but many patients will require a surgical biopsy, typically thyroid lobectomy, with intraoperative pathology. Consultation would typically be the next step in 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, because different thyroid malignancies require different surgical procedures (eg, unilateral lobectomy vs total or subtotal thyroidectomy with or without lymph node dissection) depending on several

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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.

### THYROID CANCER

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

The diagnosis of malignancy in the case of PTC is primarily based on cytologic features. If FNA in a case of PTC is indeterminate, surgical biopsy with intraoperative pathology consultation is most often diagnostic, although its efficacy and therefore its use will vary across institutions, surgeons, and pathologists.

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 most common 4 gene variants that carry the highest impact on tumor diagnosis and prognosis are *BRAF* and *RAS* single nucleotide variants (SNVs), and *RET/PTC* and *PAX8/PPAR $\gamma$*  rearrangements.

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

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

### **Molecular Diagnostic Testing**

#### ***Variant Detection and Rearrangement Testing***

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

The ThyroSeq v.2 Next-Generation Sequencing panel (CBLPath, Ocala, FL) is an NGS 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.

ThyGenX is an NGS panel that sequences 8 genes and identifies specific gene variants and translocations associated with thyroid cancer. ThyGenX is intended to be used in conjunction with the ThyraMIR microRNA expression test when the initial ThyGenX test is negative.

#### ***Gene Expression Profiling***

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

The Afirma Gene Expression Classifier (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 evaluate thyroid nodules that have an "indeterminate" classification on FNA as a method to select patients ("rule out") who are at low risk for cancer.

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

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

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**Algorithmic Testing**

Algorithmic testing involves the use of two 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, Veracyte also markets 2 “malignancy classifiers” that use mRNA expression-based classification to evaluate for *BRAF* variants (Afirma BRAF) or variants associated with medullary thyroid carcinoma (Afirma MTC). Table 1 describes the testing algorithms for Afirma MTC and Afirma BRAF.

**Table 1. Afirma MTC and Afirma BRAF Testing Algorithms**

Test 1	Test 1 Result	Reflex to Test 2
Thyroid nodule on fine needle aspirate	“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 an FNA sample with increased pretest probability for malignancy. A positive result with Afirma MTC or Afirma BRAF would inform preoperative planning such as planning for a hemi- vs a total thyroidectomy or performance of a central neck dissection.

**Algorithmic Testing Using ThyGenX and ThyraMIR**

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

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

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

### **FDA or Other Governmental Regulatory Approval**

#### **U.S. Food and Drug Administration (FDA)**

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

In 2013, the THxID™<sup>‡</sup>-BRAF kit (bioMérieux, Marcy l'Etoile, France), an in vitro diagnostic device, was approved by the FDA through the premarket approval process to assess specific *BRAF* variants in melanoma tissue via real-time PCR. However, there are currently no diagnostic tests for thyroid cancer mutation analysis with approval from the FDA.

Table 2 provides a summary of commercially available molecular diagnostic tests for indeterminate thyroid pathology.

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

Test	Methodology	Analyte(s)	Report
Afirma <sup>®</sup> GEC	mRNA gene expression	167 genes	Benign/suspicious
Afirma <sup>®</sup> BRAF	mRNA gene expression	1 gene	Negative/positive
Afirma <sup>®</sup> MTC	mRNA gene expression		Negative/positive
ThyroSeq v2	Next-generation sequencing	60+ genes	Specific gene variant/translocation
ThyGenX™ <sup>a</sup>	Next-generation sequencing	8 genes	Specific gene variant/translocation
miRInform <sup>®a</sup>	Multiplex PCR by sequence-specific probes	14 DNA variants, 3 RNA fusions	Specific gene variant/translocation
ThyraMIR™	microRNA expression	10 microRNAs	Negative/positive
RosettaGX™ Reveal	microRNA expression	24 microRNAs	<ul style="list-style-type: none"> <li>• Benign</li> <li>• Suspicious for malignancy</li> <li>• High risk for medullary carcinoma</li> </ul>

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

<sup>a</sup> The miRInform<sup>®</sup> test is the predicate test to ThyGenX™ and is not commercially available.

### **Centers for Medicare and Medicaid Services (CMS)**

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.

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

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

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

### **Rationale/Source**

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

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose.

### **MOLECULAR MARKERS TO RULE OUT MALIGNANCY**

#### **Clinical Context and Test Purpose**

The purpose of molecular testing in individuals with indeterminate findings on FNA of thyroid nodules is to rule out malignancy and eliminate the need for surgical resection.

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

The following PICOTS were used to select literature to inform this review.

#### ***Patients***

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 resection.

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### **Interventions**

The relevant intervention of interest is molecular testing which includes either Afirma Gene Expression Classifier (GEC), ThyroSeq v2, or RosettaGX Reveal.

### **Comparators**

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

### **Outcomes**

The potential beneficial outcomes of primary interest would result from avoiding an unneeded surgical biopsy resection (eg, lobectomy or hemithyroidectomy) in a true-negative thyroid nodule that is benign.

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

### **Timing**

The time frame for evaluating the performance of the test varies from the initial FNA to surgical resection to weeks to months following an indeterminate result to years. PTC is an indolent cancer, and a nodule could be observed for many years to ensure no clinical change.

### **Setting**

The primary setting would be in endocrinology.

### **Afirma GEC**

#### **Technically Reliable**

Walsh et al (2012) verified the analytic performance of the Afirma GEC in the classification of cytologically indeterminate FNAs from thyroid nodules. The analytic performance studies were designed to characterize the stability of the RNA in the aspirates during collection and shipment, the analytic sensitivity and specificity, and the assay performance studies including intranodule, intraassay, interassay, and interlaboratory reproducibility. Concordance of the GEC calls was 100% for samples tested under different shipping conditions, 97.2% across different RNA input amounts, 100% under different dilutions with normal tissue, and 96% across different genomic DNA contamination amounts. The intra-assay, interassay, interlaboratory, and intranodule concordance rates were 93.9%, 97%, 100%, and 95%, respectively. The authors concluded that the analytic sensitivity and specificity, robustness, and quality control of the GEC were successfully verified.

#### **Clinically Valid**

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 negative predictive

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value (NPV) specificity of 95% and a 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 tested separately 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 study was 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. The authors noted that 25 of those prospective clinical FNA samples were derived from the 413 samples described above.

After exclusion of the 25 used for test validation and those without a valid GEC result, 265 FNA samples were evaluated with the 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 ranged from 85% for “suspicious cytologic findings” to 95% for “atypia of undetermined clinical significance.” There were 7 FNAs with false-negative results, six of which were thought to be due to hypocellular aspirate specimens.

### Retrospective Clinical Validation

In 2014, Alexander et al reported on results from a multicenter retrospective analysis of 339 thyroid nodules that underwent Afirma GEC testing for indeterminate cytology on FNA AUS or FLUS, follicular neoplasm, or suspicious for malignancy) at 5 academic medical centers. Most nodules sent for GEC testing were AUS or FLUS or follicular neoplasm. The distribution of GEC testing results for each cytologic classification is shown in Table 3.

**Table 3. GEC Testing Results**

Cytologic Classification	N	GEC Testing Results, n (%)		
		Benign	Suspicious	Nondiagnostic
Atypia or follicular lesion of undetermined significance	165	91 (55)	66 (40)	8 (5)
Follicular neoplasm	161	79 (49)	73 (45)	9 (6)
Suspicious for malignancy	13	4 (31)	9 (69)	0

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Total	339	174	148	17
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Adapted from Alexander et al (2014).

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). On the assumption that, absent the GEC results, thyroid surgery would be recommended for patients with cytologically indeterminate FNA results, the authors reported that GEC results altered management in 50% of patients. Table 4 shows thyroidectomy biopsy results for the subset of patients in Table 3 who underwent surgery.

**Table 4. Thyroidectomy Results**

GEC Results	N	Surgery Recommended, n	Surgery Completed, n	Pathology Malignant for Those With Completed Surgery, n (%)
Suspicious	148	141	121	53 (44)
Benign	174	4	11	1 (9)

Adapted from Alexander et al (2014).

Seventeen patients who had indeterminate cytology, benign Afirma GEC results, and did not undergo surgery had follow-up beyond 1 year. Of those 17 patients, 3 patients had surgery to remove 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 an 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 conducted a meta-analysis of studies reporting on the performance of the Afirma GEC in cytologically indeterminate nodules. Seven studies met inclusion criteria, which required that studies reported on the use of the Afirma GEC in nodules found indeterminate on FNA (including AUS or FLUS; suspicious for follicular or Hürthle 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 in Table 5.

**Table 5. Pooled GEC Performance**

Outcomes	Point Estimate	95% Confidence Interval	I <sup>2</sup>
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	

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Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

Diagnostic odds ratio 7.9 4.1 to 15.1

Adapted from Santhanam et al (2016).

Retrospective single-center studies, including Harrell and Bimston (2014), Lastra et al (2014), Mclver et al (2014), and Yang et al (2016), have reported the diagnostic accuracy of the Afirma GEC (see Table 6). All studies were subject to ascertainment bias because a large proportion of individuals with Afirma benign reports did not undergo surgery, which made determining the sensitivity and specificity of the GEC assay impossible. However, the rates of malignancy among patients with Afirma benign results who did undergo surgery were 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. One additional publication (Celik et al, 2015) reported on Afirma GEC testing; however, included in this publication were individuals with benign and suspicious cytology on FNA—and those individuals are not necessarily considered to be part of the “target population.”

**Table 6. Single-Center Studies Reporting Afirma GEC Results**

Study (Year)	Indeterminate FNA Samples, n (%)	Afirma GEC Test Result	N	With Thyroidectomy, n	With Malignancy on Thyroidectomy, n
Harrell and Bimston (2014)	58 AUS/FLUS or FN	Suspicious	36 <sup>a</sup>	30	21
		Benign	20	5	2
Lastra et al (2014)	69 (51.5) AUS/FLUS 39 (29.5) FN 25 (19) FNOF	Suspicious	62	48	22
		Benign	70	2	0
Mclver et al (2014)	12 (11.4) AUS/FLUS 93 (88.6) FN/HCN	Suspicious	44 <sup>b</sup>	32	5
		Benign	16	4	1
Yang et al (2016)	165 (76) AUS/FLUS 24 (11) SFN/FN	Suspicious	80	62	32
		Benign	94	5	0
Witt et al (2016)	47 AUS/FLUS or SFN/FN (32 with GEC attempted <sup>c</sup> )	Suspicious	15	15	6
		Benign	14	0	NA; 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: Hürthle cell neoplasm; NA: not applicable; SFN: suspicious for follicular neoplasm.

<sup>a</sup>Two samples inadequate due to low mRNA content.

<sup>b</sup>GEC results were available for 60 subjects.

<sup>c</sup>Three samples were inadequate.

There are limited data on the true-negative rates 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 have reported 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 who had cytologically benign nodules. A total of 95 cytologically indeterminate and Afirma GEC benign nodules in 90 patients

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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 postdiagnosis. 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 and Afirma GEC benign nodules (13.8% vs 0.9%,  $p<0.001$ ).

### ***Clinically Useful***

No evidence directly demonstrating improved outcomes in patients managed with the Afirma GEC was identified. Therefore, a chain of evidence was developed, which addresses 2 key questions:

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

### ***Changes in Management***

The clinical setting in which the Afirma GEC is meant to be used is well-defined: individuals with AUS or FLUS or follicular neoplasm or who are suspicious for follicular neoplasm (SFN) on FNA who do not have other indications for thyroid resection (ie, in whom the GEC results would play a role in surgical decision making).

Decision impact studies, most often reporting on clinical management changes but not on outcomes after surgical decisions were made, have suggested that, in at least some cases, surgical decision making changed. 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 resect 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.

Sipos et al (2016) performed a retrospective study of nonacademic medical practices using the Afirma GEC to determine the long-term nonoperative rate of thyroid nodules with benign results. Of the patients with Afirma benign results during 36 months of follow-up, 17.3% underwent surgery. Eighty-eight percent of all surgeries were performed within the first 2 years after a benign Afirma GEC result.

The 2014 study by Alexander et al provided evidence on clinical management changes for patients with indeterminate thyroid nodules tested with 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.

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Two studies (Aragon Han et al [2014], Noureldine et al [2015]) evaluated the potential for the Afirma GEC test to change surgical decision making by comparing actual surgical decision making when 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 (10%-15%).

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 postimplementation of Afirma GEC. A total of 158 FNAs were sent for Afirma GEC with 73 suspicious and 8 benign Afirma cases going for 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 expert thyroid cytopathology and Afirma GEC in a community practice. Historical data were compared with data after implementation of the FNA protocol. Prior to protocol implementation, the rates of indeterminate cytology and diagnostic surgery were 26% and 24%. After protocol implementation, 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.

### **RosettaGX Reveal**

#### ***Technically Reliable***

Benjamin et al (2016) reported on the analytic performance of the RosettaGX Reveal in the classification of cytologically indeterminate thyroid FNA smears from routinely prepared cytology slides. The analytic sensitivity of the assay was determined to be  $1.28 \times 10^{-2}$  ng. The assay was reported to classify FNA smears with low amounts of thyroid cells. The interlaboratory agreement for samples extracted in a single laboratory and processed at 2 different laboratories was 92.68%. For samples that were extracted and complementary DNA synthesis run in a single laboratory with subsequent quantitative real-time PCR (rt-PCR) run in 2 different laboratories, the interlaboratory agreement was 90.9%.

#### ***Clinically Valid***

Lithwick-Yanai et al (2017) described the development and initial clinical validation of using the RosettaGX Reveal quantitative rt-PCR assay for 24 microRNAs in a multicenter, retrospective cohort study using 201 FNA smears. The results of the clinical validation study are reported in Table 7.

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**Table 7. RosettaGX Reveal Performance**

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

Adapted from Lithwick-Yanai et al (2017).  
 QC: quality control.

No prospective clinical studies for RosettaGX™+ Reveal were identified.

### **Clinically Useful**

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

### *Improved Outcomes*

A simplified decision model was developed for use with Afirma GEC in individuals with cytologically indeterminate FNA samples. It is shown in Appendix Figure 1. 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 the standard care plan, some patients without cancer will have avoided a biopsy, which is weighed against the small increase in missed cancers in patients who had cancer but tested as Afirma benign.

Assuming that the rate of cancer in cytologically indeterminate thyroid nodules is approximately 20%, 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 a 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 amenable to ongoing surveillance (clinical, ultrasound, and with repeat FNAs), with minimal morbidity.

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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.

### **Section Summary: Molecular Markers to Rule Out Malignancy**

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 GEC testing in cytologically indeterminate lesions, but with only a single study with the marketed test reporting a true NPV, the clinical validity is uncertain. For the RosettaGX Reveal test, an analytic validation study and a retrospective clinical validation have been reported. No prospective studies for patients managed with the RosettaGX Reveal were identified, so the clinical validity remains uncertain.

### **MOLECULAR MARKERS TO RULE IN MALIGNANCY**

#### **Clinical Context and Test Purpose**

The purpose of testing for molecular markers (eg, SNVs and gene rearrangements) in individuals with indeterminate findings on FNA of thyroid nodules is to rule in malignancy and to guide surgical approach or management.

The relevant question addressed in this evidence review is: Does testing for molecular markers predict malignancy and alter surgical approach or management and lead to improved health outcomes?

The following PICOTS were used to select literature to inform this review.

#### **Patients**

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

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### **Interventions**

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

### **Comparators**

The relevant comparator of interest is standard surgical management through surgical resection, including a 2-stage surgical biopsy (ie, lobectomy) followed by definitive surgery (ie, hemithyroidectomy or thyroidectomy).

### **Outcomes**

The potential beneficial outcomes of primary interest are to allow for appropriate surgical planning in the preoperative period (eg, 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 testing would mitigate any risk of inappropriately performing more extensive thyroidectomy in the absence of malignancy.

### **Timing**

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

### **Setting**

The primary setting would be in endocrinology.

## **Gene Expression Classifiers to Predict Malignancy**

### **Technically Reliable**

In 2015, Diggans et al described the development and validation of the Afirma BRAF malignancy classifier. The study included FNA biopsies from 716 thyroid nodules. Biopsies were evaluated with quantitative PCR for the *BRAF* V600E gene, with 181 used as a training sample and 535 used as a validation sample. The Afirma BRAF malignancy classifier was generated using robust multichip average-normalized gene expression summaries, and the classifiers were evaluated for positive percent agreement (PPA) and negative percent agreement with the PCR-derived gene classification. The highest scoring classification method and gene set were then used in a final round of model building. The maximum PPA and negative percent agreement for all cytology categories were observed when the threshold for *BRAF*-positive status was 5% or more *BRAF* variants. At 5% analytic sensitivity, Afirma BRAF demonstrated a PPA with PCR

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results of 90.4% (95% CI, 83.5% to 95.1%) and a negative percent agreement of 99.0% (95% CI, 97.6% to 99.7%). Two samples in the training set and 4 samples in the validation set were Afirma BRAF–positive but negative (0% variant) on PCR, which the authors attributed to technical variability in either assay or to variants other than the *BRAF* V600E variant that cause similar gene expression changes.

Intra- and interrater reproducibility of the classifier were evaluated using 9 FNA biopsies and 3 tissue controls; the biopsies and tissue controls were selected from training samples with high (*BRAF*-positive) or low (*BRAF*-negative) classifier scores and scores near the classifier decision boundary. Each FNA biopsies and tissue was processed from total RNA in triplicate in each of 3 different runs across days, operators, and reagent lots. The intraassay standard deviation (SD) of Afirma BRAF scores was 0.171 (95% CI, 0.146 to 0.204). Of the 106 Afirma BRAF calls produced (2 arrays failed quality control requirements), 106 resulted in concordant calls across all 3 runs (100% concordance). The interassay SD of scores was 0.204 (95% CI, 0.178 to 0.237) for scores measured on a 6-point scale. These results suggest low intra- and interrater variability.

In 2016, Kloos et al described the development of the Afirma MTC classifier in a study that also described the clinical validity of the MTC classifier.

Pankratz et al (2016) studied the analytic performance of Afirma MTC classifier from fresh-frozen tissue specimens with a confirmed medullary thyroid carcinoma (MTC) diagnosis. Twenty-seven MTC tissue specimens were compared with 20 deidentified FNA samples from normal donors. The reported clinical sensitivity of the Afirma MTC classifier was 96.3% (95% CI, 81.0% to 99.9%).

### **Clinically Valid**

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

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 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 for GEC testing (the Afirma GEC described below). In this sample, 43 cases were Afirma MTC–positive, of which 42 were considered to be clinically consistent with MTC on pathology or biochemical testing, for a positive predictive value (PPV) of 97.7% (95% CI, 86.2% to 99.9%).

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### **Clinically Useful**

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

In a retrospective analysis, Yip et al (2014) reported on outcomes after implementation of an algorithm incorporating molecular testing of thyroid FNA samples to guide the extent 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 SNVs; *RET/PTC1*, *RET/PTC3*, and *PAX8/PPAR $\gamma$*  rearrangements) was prospectively obtained for all FNAs with indeterminate cytology (FLUS, follicular neoplasm, suspicious for malignancy), and for selective FNAs at the request of the managing physician for selected nodules with benign or nondiagnostic cytology. The study also included a second cohort of patients who did not have molecular testing results available. For patients treated with molecular diagnosis, a positive molecular diagnostic test was considered an indication for an initial total thyroidectomy. Patients with FLUS and negative molecular diagnostic results were followed with repeat FNA, followed by 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 and 349 without molecular diagnostics. Positive molecular testing results were obtained in 56 (17% of those managed with molecular diagnostics) patients, most commonly *RAS* variants (42/56 [75%]), followed by *BRAF* V600E (10/56 [18%]) and *BRAF* K601E (2/56 [4%]) variants, and *PAX8/PPAR $\gamma$*  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 (ie, 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 diagnostic of thyroid cancer of 89% and 27%, respectively, 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%.

In 2015, a task force from the American Thyroid Association published a review with recommendations for the surgical management of FNA-indeterminate nodules using various molecular genetic tests. This review reported on the estimated likelihood of malignancy in an FNA-indeterminate nodule depending on results of

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the Afirma GEC test (described above) and other panels designed to rule in malignancy. Depending on the estimated prebiopsy likelihood of malignancy, recommendations for surgery included observation, active surveillance, repeat FNA, diagnostic lobectomy, or oncologic thyroidectomy.

### **Section Summary: Molecular Markers to Predict Malignancy**

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 higher certainty on FNA if such testing permits better preoperative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. A statement from the American Thyroid Association provides some guidelines for surgeons managing patients with indeterminate nodules. However, adoption of these guidelines in practice and outcomes associated with them are uncertain.

### **MOLECULAR MARKERS TO RULE OUT AND RULE IN MALIGNANCY**

#### **Clinical Context and Test Purpose**

The purpose of the ThyroSeq v2 test and the combined ThyGenX Thyroid Oncogene Panel and ThyraMIR microRNA classifier in individuals with indeterminate findings on FNA(s) of thyroid nodules is to predict malignancy and inform surgical planning decisions with positive results using ThyroSeq v2 or the ThyGenX, and if negative, to predict benignancy using ThyraMIR microRNA classifier to eliminate or necessitate the need for surgical biopsy and guide surgical planning.

The relevant question addressed in this evidence review is: Does the ThyroSeq v2 test or the combined use of ThyGenX and ThyraMIR appropriately eliminate or necessitate the need for surgical resection or biopsy and lead to improved health outcomes?

The following PICOTS were used to select literature to inform this review.

#### **Patients**

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

#### **Interventions**

The relevant interventions of interest are either: (a) the ThyroSeq v2 test; or (b) the combined ThyGenX Thyroid Oncogene Panel and ThyraMIR microRNA classifier testing.

#### **Comparators**

The relevant comparator of interest is surgical biopsy and/or standard surgical management through surgical resection.

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### **Outcomes**

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

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

### **Timing**

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

### **Setting**

The primary setting would be in outpatient endocrinology.

### **ThyroSeq v2 Test**

#### ***Technically Reliable***

SNVs in specific genes associated with thyroid cancer (eg, the *BRAF* V600E gene) and the detection of genetic rearrangements associated with thyroid cancer (eg, the *RET/PTC* rearrangements) are typically detected with Sanger sequencing or next-generation sequencing (NGS) methods. In the case of testing for gene variants associated with thyroid cancer malignancy, analytic validity refers to a test's technical accuracy in detecting a variant that is present or in excluding a variant that is absent. The RT-PCR-based methods are generally considered to have high accuracy. For example, Smith et al (2014) reported on the technical performance characteristics for *BRAF* variant detection by qualitative PCR in thyroid FNA samples with high within- and between-run reproducibility.

NGS is expected to have high accuracy for detecting a variant that is present. However, with increasing numbers of tested variants, there is increased risk of detection of variants of uncertain significance. The variants of uncertain significance rate for currently available NGS panels for thyroid cancer is not well-characterized. Nikiforova et al (2013) described the development and validation of a multigene NGS panel for thyroid cancer, the ThyroSeq panel. They developed a custom library of gene sequence variants based on variants previously reported in the literature. The assay demonstrated 100% accuracy in evaluating samples of 15 thyroid tumors and 3 cell lines with known genetic alterations and 15 DNA samples with no variants. In analysis of 229 DNA samples from frozen tissues (n=105), formalin-fixed, paraffin-embedded (FFPE) tissues (n=72), and FNAs (n=52), the panel identified variants in 19 (70%) of 27 of classic PTCs, 25 (83%) of 30 follicular variant PTCs, 14 (78%) of 18 conventional, and 7 (39%) of 18 Hürthle cell carcinomas, 3 (30%) of 10 poorly differentiated carcinomas, 20 (74%) of 27 anaplastic thyroid carcinomas, and 11 (73%) of 15 MTCs. Of 83 benign nodules, 5 (6%) were positive for variants.

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# Louisiana

## Molecular Markers in Fine Needle Aspirates of the Thyroid

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Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

### ***Clinically Valid***

A number of studies have evaluated whether testing for SNVs or gene fusions (either SNVs or panels) 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.

### *Variants Association With Malignancy*

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%;  $I^2=77%$ ).

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 the results with final histology after surgical resection. Sixteen studies analyzed a single variant (eg, *BRAF* variant or *RET/PTC* rearrangement) and four analyzed a panel of 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%), a mean false-positive rate of 1.25% (range, 0%-4%), and a mean false-negative rate of 9% (range, 1%-21%). Based on 2 studies that examined *RET/PTC* rearrangements, 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.

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 detailed below; 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 and rearrangements (*BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARY*) in 470 FNA samples of thyroid nodules from 328 consecutive patients. Variant status 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 completing the cytologic evaluation; preoperative cytologic

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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 and rearrangements were found (18 *BRAF*, 8 *RAS*, 5 *RET/PTC*, 1 *PAX8/PPAR $\gamma$* ); after surgery, 31 (97%) variant-positive nodules were diagnosed as malignant on pathologic examination and 1 (3%) as a 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. 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 variant screening in 117 FNA samples classified as AUS or FLUS. *BRAF*, *RAS*, *RET/PTC*, or *PAX8/PPAR $\gamma$*  variants and rearrangements 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 variant detected was 7.6%.

In 2011, Nikiforov et al reported on 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 AUS or FLUS; 247 follicular or Hürthle cell neoplasms or SFNs; 67 suspicious for malignant cells). Eighty-seven *BRAF*, *RAS*, *RET/PTC*, or *PAX8/PPAR $\gamma$*  variants and rearrangements were detected. At 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%, and 68% for samples with AUS or FLUS, follicular or Hürthle cell neoplasms or SFNs, and suspicious for malignant cells on cytology, respectively), but a high specificity (99%, 97%, 96%, respectively). The NPVs for the variant analysis results were 94%, 86%, and 72% for samples with AUS or FLUS, follicular or Hürthle cell neoplasms or SFN, 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 complete thyroidectomy as a first surgery.

In a subsequent study, Nikiforov et al (2014) evaluated the accuracy of an NGS panel that included tests for SNVs in 13 genes and for 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 Hürthle cell neoplasm or suspicious for follicular or Hürthle 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, respectively. In the retrospective cohort, of the 25 malignant nodules, 22 were PTCs, and 3 were follicular thyroid carcinomas (FTCs). In the

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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 Table 8.

**Table 8. Performance of ThyroSeq Panel**

Variant Testing Outcomes	Nikiforov et al (2014)		Overall (N=143)	Nikiforov et al (2015)
	Retrospective (n=91)	Prospective (n=52)		Known Outcome (n=98)
Negative	64 (2 cancer; 62 benign)	37 (2 cancer; 35 benign)		72 (2 cancer; 70 benign)
Positive	27 (23 cancer; 4 benign)	15 (12 cancer; 3 benign)		26 (20 cancer; 6 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 PPV. In this case, they proposed that the panel could be used to both “rule in” and “rule out” invasive cancers.

The same group (Nikiforov et al) reported on 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 AUS or 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. Two other 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 gene testing in indeterminate thyroid nodules are summarized in Table 9. (In some cases, measures of agreement were calculated from data provided in the published article.)

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

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

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Study (Year)	Population	Genes and Rearrangements Tested	Insufficient or Inadequate for Analysis	Measures of Agreement				
				Sen	Spec	PPV	NPV	Acc
Ohuri et al (2010)	100 patients with 117 atypia or follicular lesions of undetermined significance	<i>NTRK1</i> <i>BRAF, NRAS, HRAS, KRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ</i>	NR	60	100	100	92	93
Cantara et al (2010)	41 indeterminate and 54 suspicious thyroid nodules	<i>BRAF, H-K-NRAS, RET/PTC, TRK, PAX8-PPARγ</i>	53	86 <sup>a</sup> 80 <sup>b</sup>	97 <sup>a</sup> 100 <sup>b</sup>	86 <sup>a</sup> 100 <sup>b</sup>	97 <sup>a</sup> 47 <sup>b</sup>	95 <sup>a</sup> 83 <sup>b</sup>
Xing et al (2009)	25 indeterminate, dominant nodules	<i>BRAF</i>	NR	14	100	100	48	52
Jara et al (2015)	66 nodules suspicious for PTC	<i>BRAF</i>	NR	46	88	88	44	61
Rossi et al (2015)	140 indeterminate or suspicious for malignancy or malignant nodules	<i>BRAF</i>	NR	90 <sup>c</sup> 50 <sup>d</sup> 40 <sup>e</sup>	100 <sup>c</sup> 100 <sup>d</sup> 100 <sup>e</sup>	100 <sup>c</sup> 100 <sup>d</sup> 100 <sup>e</sup>	93 <sup>c</sup> 69 <sup>d</sup> 14 <sup>e</sup>	96 <sup>c</sup> 77 <sup>d</sup> 46 <sup>e</sup>
Beaudenon-Huibregtse et al (2014)	53 nodules with indeterminate/nondiagnostic FNA	<i>BRAF, HRAS, KRAS, NRAS, PAX8-PPARγ, RET-PTC1, RET-PTC3</i>		48	89	81	64	
Valderrobano et al (2017)	190 indeterminate thyroid nodules	ThyroSeq v2 (60+ genes)	2	70	77	42	91	

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

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

Mathur et al (2010) 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 Hürthle cell neoplasms, and 4% were suspicious for malignancy. On final analysis, 165 nodules were benign and 123 malignant. In the 423 FNA samples, 24 *BRAF* V600E, 7 *KRAS*, and 21 *NRAS* variants, and 4 *PAX8-PPARγ*, 3 *RET/PTC1*, and 2 *RET/PTC3* rearrangements were

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detected. In all, 17 (10.3%) of 165 benign thyroid nodules had a variant or rearrangement compared with 26% (32/123) malignant tumors ( $p < 0.05$ ).

Eszlinger et al (2014) retrospectively analyzed a panel of variants (*BRAF* and *RAS* SNVs, *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, 89 benign on FNA). Forty-seven 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 a specificity of 90.4% for detecting malignancy, with a PPV of 77.2% and NPV of 89.4%. The presence of a *BRAF* variant or a *RET/PTC* rearrangement was associated with cancer in 100% of samples.

The association between *BRAF* variants and PTC is supported in a 2015 report by Park et al on 294 patients with thyroid nodules whose FNA samples were evaluated for *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 were 80.2% (95% CI, 71.9% to 86.9%) and 76.9% (95% CI, 68.3% to 84.0%), respectively.

### *Genetic Variants Association With Tumor Behavior*

As noted, the presence of *BRAF* variants is strongly associated with malignancy in thyroid nodule FNA samples. *BRAF* variants have also been associated with more aggressive clinicopathologic features in individuals diagnosed with PTC.

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

Xing et al (2009) investigated the utility of *BRAF* variant testing of thyroid FNA specimens for preoperative risk stratification of PTC in 190 patients. A *BRAF* variant in preoperative FNA specimens was associated with poorer clinicopathologic outcomes for PTC. Compared with the wild-type allele, a *BRAF* variant strongly predicted extrathyroidal extension (23% vs 11%;  $p = 0.039$ ), thyroid capsular invasion (29% vs 16%;  $p = 0.045$ ), and lymph node metastasis (38% vs 18%;  $p = 0.002$ ). During a median follow-up of 3 years (range, 0.6-10 years), PTC persistence or recurrence was seen in 36% of *BRAF* variant-positive patients and 12% of *BRAF* variant-negative patients, with an odds ratio of 4.16 (95% CI, 1.70 to 10.17;  $p = 0.002$ ). The PPV

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and NPV for preoperative FNA-detected *BRAF* variant to predict PTC persistence or recurrence were 36% and 88%, respectively, for all histologic subtypes of PTC. The authors concluded that preoperative *BRAF* variant testing of FNA specimens might provide a novel tool to preoperatively identify PTC patients at higher risk for extensive disease (extrathyroidal extension and lymph node metastases) and those more likely to manifest disease persistence or recurrence.

**ThyGenX Thyroid Oncogene Panel and ThyraMIR microRNA Classifier**  
**Technically Reliable**

Hadd et al (2013) reported on targeted NGS of cancer genes in 38 FFPE and 10 FNA tumor specimens. The results showed an accuracy rate of 96.1% (95% CI, 96.1% to 99.3%) compared with Sanger sequencing; Sanger sequencing has an analytic sensitivity of approximately 15% to 20%. When NGS was compared with a multiplex detection system with a 1% variant detection rate, the accuracy was reported to be 99.6% (95% CI, 97.9% to 99.9%).

Wylie et al (2016) reported on the development of the ThyraMIR miRNA classifier, along with a 17-variant oncogene panel including *BRAF*, *RAS*, *RET*, or *PAX*. An miRNA classifier was originally developed using rt-PCR methodology in a sample of 257 surgical specimens, and validated in an independent set of 42 nodules with indeterminate cytology. A 17-variant panel covering validated oncogenic gene alterations for *BRAF*, *RAS*, *RET*, or *PAX8* genes was tested on preoperative FNA and surgical specimens. Optimization of miRNA classifiers A and B resulted in the commercial ThyraMIR Classifier. ThyraMIR was used on a subset of thyroid tissues negative by the targeted 17-variant panel and resulted in a sensitivity of 85% and specificity of 95%.

*Section Summary: Technically Reliable*

The analytic validity of targeted NGS of cancer genes is expected to be high. Concordance rates between Sanger sequencing and NGS are high but limited lower analytic sensitivity of Sanger sequencing. Concordance rates increased when NGS is compared with an orthogonal technology with a 1% variant detection rate. One study describing the development of a miRNA classifier was identified; a description of the analytic validity of the corresponding commercially available NGS version of the oncogene pane has not been identified but is expected to be high.

**Clinically Valid**

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

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

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

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FN/SFN (95% CI), %	51	82 (57 to 96)	91 (76 to 98)	82 (57 to 96)	91 (76 to 98)	48 (9 to 269)
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AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspiration; NPV: negative predictive value; PPV: positive predictive value; SFN: suspicious for a follicular neoplasm.

### *Section Summary: Clinically Valid*

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

### **Clinically Useful**

Direct evidence for the clinical utility for the ThyroSeq v2 test and the combined ThyGenX and ThyraMIR diagnostic testing algorithm is lacking. In the absence of direct evidence for the clinical utility of the combined testing, a chain of evidence may be constructed to infer potential clinical utility of the combined diagnostic testing algorithm. No studies using ThyGenX NGS panel in FNA samples were identified. However, available evidence has suggested that use of variant testing using NGS in thyroid FNA samples is generally associated with 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 an NPV sufficiently high enough to identify which patients can undergo active surveillance over thyroid surgery. In the diagnostic algorithm that reflexes to the ThyraMIR after a negative ThyGenX result, patients receiving reflex testing could identify who may undergo active surveillance over thyroid surgery. A single study using a 17-variant panel with ThyraMIR showed a NPV of 94%. Therefore, the high NPV of ThyraMIR has the potential to accurately predict benignancy and triage patients to active surveillance.

### *Section Summary: Clinically Useful*

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

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### 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 and to avoid surgical biopsy, the evidence includes a prospective clinical validity study with the Afirma GEC and a chain of evidence to support clinical utility. Relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. In a multicenter validation study, the Afirma GEC was reported to have a 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 both 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 patients who are benign, but the exact NPV is unknown. The available evidence suggests that the decisions a physician makes regarding surgery are altered by GEC results; however, it should be noted that 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 true NPV, the clinical validity is uncertain. For the RosettaGX Reveal test, no prospective clinical studies were identified. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular markers to rule in malignancy and to guide surgical planning, the evidence includes prospective and retrospective studies of clinical validity. Relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. Variant analysis has the potential to improve the accuracy of an equivocal FNA of the thyroid and may play a role in preoperative risk stratification and surgical planning. Single-center studies have suggested that testing for a panel of genetic variants associated with thyroid cancer may allow for the appropriate selection of patients for surgical management with an initial complete thyroidectomy. Prospective studies in additional populations are needed to validate these results. Variant analysis does not achieve an NPV sufficiently high enough 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.

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 to rule in surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq v2 test and 2 retrospective clinical validation studies that utilized a predicate test 17-variant panel (*miRInform*) test to the current ThyGenX and ThyraMIR. Relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. In a retrospective validation study on FNA samples, the 17-variant panel (*miRInform*) test and ThyraMIR had a sensitivity of 89%, and a NPV of 94%. Pooled retrospective and prospective clinical validation studies of ThyroSeq v2 have reported a combined NPV of 96% and a positive predictive value of 83%. No studies were identified demonstrating the diagnostic characteristics of the marketed ThyGenX. No studies were identified demonstrating evidence of direct

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Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

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.

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Policy # 00332

Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

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Policy # 00332

Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

Returned to Active Status: 02/21/2018

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### **Policy History**

Original Effective Date: 12/19/2012

Current Effective Date: 02/21/2018

02/01/2018 Medical Policy Committee review

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Policy # 00332  
 Original Effective Date: 12/19/2012  
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 Returned to Active Status: 02/21/2018

02/21/2018 Medical Policy Implementation Committee approval. Returned to active status.  
 03/15/2018 Coding update  
 Next Scheduled Review Date: 02/2019

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