Preauthorization is required.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient’s contract at the time the services are rendered.

### Populations

<table>
<thead>
<tr>
<th>Individuals:</th>
<th>With thyroid nodule(s) and indeterminate findings on fine needle aspirate</th>
</tr>
</thead>
</table>

### Interventions

| Interventions of interest are: | Fine needle aspirate sample testing with molecular markers to rule out malignancy and to avoid surgical biopsy |

### Comparators

| Comparators of interest are: | Surgical biopsy |

### Outcomes

| Relevant outcomes include: | Disease-specific survival, Test accuracy, Test validity, Morbid events, Resource utilization |

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### Interventions

| Interventions of interest are: | Fine needle aspirate sample testing with molecular markers to rule in malignancy and to guide surgical planning |

### Comparators

| Comparators of interest are: | Surgical management based on clinicopathologic risk factors |

### Outcomes

| Relevant outcomes include: | Disease-specific survival, Test accuracy, Test validity, Morbid events, Resource utilization |

### Populations

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</tr>
</thead>
</table>

### Interventions

| Interventions of interest are: | Fine needle aspirate sample testing with molecular markers to rule out or to rule in malignancy for surgical planning |

### Comparators

| Comparators of interest are: | Surgical management based on clinicopathologic risk factors and/or surgical |

### Outcomes

| Relevant outcomes include: | Disease-specific survival, Test accuracy, Test validity, Morbid events, Resource utilization |

**Description**

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

**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 two 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 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.

Policy

The use of the either Afirma Gene Expression Classifier or ThyroSeq v2 in fine-needle aspirates of thyroid nodules with indeterminate cytologic findings (i.e., Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) may be considered to be medically necessary in patients who have the following characteristics:

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

The use of 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 two stage surgical biopsy followed by definitive surgery may be considered medically necessary:

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

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

Policy Guidelines

In patients who do not undergo surgical biopsy or thyroidectomy on the basis of gene expression classifier 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 one 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

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated variant</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td>Variant</td>
<td></td>
<td>Change in the DNA sequence</td>
</tr>
<tr>
<td>Familial variant</td>
<td></td>
<td>Disease-associated variant identified in a proband for use in subsequent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>targeted genetic testing in first-degree relatives</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely Pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain significance</td>
<td>Change in DNA sequence with uncertain effects on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
</tbody>
</table>
Variant Classification | Definition
--- | ---
Benign | Benign change in the DNA sequence

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

**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.

**Medicare Advantage**

For Medicare Advantage Afirma will be considered **medically necessary** for 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
- Patients with an indeterminate follicular pathology on fine needle aspiration.

**Medicare Advantage Policy Guidelines**

This test is expected to be necessary once per patient lifetime. Should the unlikely situation of a second, unrelated thyroid nodule with indeterminate pathology occur, medical necessity may be considered with support documentation.

**Background**

**Thyroid Nodules**

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

**Diagnosis**

Sampling thyroid cells by 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: non-diagnostic; 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 (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.

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 four 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/PPARγ 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/PPARγ rearrangements. These variants have been identified in 70% to 75% of
follicular carcinomas. Genetic alterations involving the PI3K/AKT signaling pathway also occur in thyroid tumors, although they are rare in well-differentiated thyroid cancers and have a higher prevalence in less differentiated thyroid carcinomas. Additional variants known to occur in poorly differentiated and anaplastic carcinomas involve the TP53 and CTNNB1 genes. Medullary carcinomas, which can be familial or sporadic, frequently possess SNVs located in the T gene.

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/PPARγ rearrangements.

The ThyroSeq v2 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 eight 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 (e.g., Barros-Filho et al [2015], Zheng et al [2015]); they are not addressed in this protocol.

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

**ALGORITHMIC TESTING**

Algorithmic testing involves the use of 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 two “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

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Test 1 Result</th>
<th>Reflex to Test 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid nodule on fine needle aspirate</td>
<td>“Intermediate”</td>
<td>Afirma MTC</td>
</tr>
<tr>
<td>Afirma GEC</td>
<td>“Malignant” or “suspicious”</td>
<td>Afirma MTC</td>
</tr>
<tr>
<td>Afirma GEC</td>
<td>“Suspicious”</td>
<td>Afirma BRAF</td>
</tr>
</tbody>
</table>

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 one 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 eight 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.

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

Regulatory Status

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Thyroid variant testing and gene expression classifiers are available under the auspices of the CLIA. Laboratories that offer LDTs must be licensed by the 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.

In 2013, the THxID™-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**

<table>
<thead>
<tr>
<th>Test</th>
<th>Methodology</th>
<th>Analyte(s)</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma® GEC</td>
<td>mRNA gene expression</td>
<td>167 genes</td>
<td>Benign/suspicious</td>
</tr>
<tr>
<td>Afirma® BRAF</td>
<td>mRNA gene expression</td>
<td>one gene</td>
<td>Negative/positive</td>
</tr>
<tr>
<td>Afirma® MTC</td>
<td>mRNA gene expression</td>
<td></td>
<td>Negative/positive</td>
</tr>
<tr>
<td>ThyroSeq v2</td>
<td>Next-generation sequencing</td>
<td>60+ genes</td>
<td>Specific gene variant/ translocation</td>
</tr>
<tr>
<td>ThyGenX™</td>
<td>Next-generation sequencing</td>
<td>eight genes</td>
<td>Specific gene variant/ translocation</td>
</tr>
<tr>
<td>miRInform®</td>
<td>Multiplex PCR by sequence-specific probes</td>
<td>14 DNA variants, three RNA fusions</td>
<td>Specific gene variant/ translocation</td>
</tr>
<tr>
<td>ThyraMIR™</td>
<td>microRNA expression</td>
<td>10 microRNAs</td>
<td>Negative/positive</td>
</tr>
<tr>
<td>RosettaGX™</td>
<td>microRNA expression</td>
<td>24 microRNAs</td>
<td>• Benign</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Suspicious for malignancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• High risk for medullary carcinoma</td>
</tr>
</tbody>
</table>

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

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

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. *For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.*

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced procedures. *Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.*

**References**

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.


