Protocol

Molecular Markers in Fine Needle Aspirates of the Thyroid

(20478)

Medical Benefit		**Effective Date**: 04/01/19	**Next Review Date**: 11/19
Preauthorization	Yes	**Review Dates**: 07/15, 07/16, 11/16, 11/17, 03/18, 11/18	

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	Interventions	Comparators	Outcomes
Individuals: • With thyroid nodule(s) and indeterminate findings on fine needle aspirate	Interventions of interest are: • Fine needle aspirate sample testing with molecular markers to rule out malignancy and to avoid surgical biopsy or resection	Comparators of interest are: • Surgical biopsy	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity • Morbid events • Resource utilization
Individuals: • With thyroid nodule(s) and indeterminate findings on fine needle aspirate	Interventions of interest are: • Fine needle aspirate sample testing with molecular markers to rule in malignancy and to guide surgical planning	Comparators of interest are: • Surgical management based on clinicopatho-logic risk factors	Relevant outcomes include: • Disease-specific survival • Test accuracy • Test validity • Morbid events • Resource utilization
Individuals: • With thyroid nodule(s) and indeterminate findings on fine needle aspirate	Interventions of interest are: • Fine needle aspirate sample testing with molecular markers to rule out or to rule in malignancy for surgical planning	Comparators of interest are: • Surgical management based on clinicopatho-logic risk factors and/ or surgical biopsy	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 or resection, 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 Gene Expression Classifier (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 [2010]), 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. The 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 avoid surgical biopsy or to rule in malignancy for surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq v2 or v3 test and two retrospective clinical validation studies that used 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 an NPV of 94%. Pooled retrospective and prospective clinical validation studies of ThyroSeq v2 have reported a combined NPV of 96% and positive predictive value of 83% in studies conducted at the institution developing the test but poorer performance at external institutions. 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 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 and single-gene telomerase reverse transcriptase (TERT) testing, are considered **investigational**.

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 one surgery.

GENETICS NOMENCLATURE UPDATE

The Human Genome Variation Society 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). The Society's nomenclature is recommended by the Human Variome Project, the HUman Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to 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

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

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.

MEDICARE ADVANTAGE

For Medicare Advantage Afirma, ThyraMIR, ThyGenX, and Thyroseq will be considered **medically necessary** for the following conditions:

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

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 surgically when detected early.

Diagnosis

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

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

Management

There is some individualization of management for patients with FNA-indeterminate nodules, but many patients will require a surgical biopsy, typically thyroid lobectomy, with intraoperative pathology. Consultation would typically be the next step in diagnosis. Approximately 80% of patients with indeterminate cytology undergo surgical resection; postoperative evaluation has revealed a malignancy rate ranging from 6% to 30%, making this a clinical process with very low specificity.[2] Thus, if 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. In 2016, reclassification of encapsulated follicular-variant PTC as a noninvasive follicular tumor with papillary-like nuclei was proposed and largely adopted; this classification removes the word carcinoma from the diagnosis to acknowledge the indolent behavior of these tumors.[3]

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

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

Genetic Variants Associated With Thyroid Cancer

A number of genetic variants have been discovered in thyroid cancer. The most common four gene variants are BRAF and RAS single nucleotide variants (SNVs) and RET/PTC and PAX8/PPARγ rearrangements.

Papillary carcinomas carry SNVs of the BRAF and RAS genes, as well as RET/PTC and TRK rearrangements, all of which can activate the mitogen-activated protein kinase pathway.[4] These mutually exclusive variants are found in more than 70% of papillary carcinomas.[4] 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.[4] 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.[4] Additional variants known to occur in poorly differentiated and anaplastic carcinomas involve the TP53 and CTNNB1 genes. Medullary carcinomas, which can be familial or sporadic, frequently possess SNVs located in the RET gene.

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

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

Molecular Diagnostic Testing

Variant Detection and Rearrangement Testing

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 v.2 Next-Generation Sequencing panel (CBLPath) is an NGS panel of more than 60 genes. According to the CBLPath's website, the test is indicated when FNA cytology suggests atypia of uncertain significance or follicular lesion of undetermined significance, follicular neoplasm or suspicious for follicular neoplasm, or suspicious for malignancy.[15] In particular, it has been evaluated in patients with follicular neoplasm and/or suspicious for follicular neoplasm on FNA as a test to increase both sensitivity and specificity for cancer diagnosis.

ThyGenX is an NGS panel that sequences 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 available and stratify tissue from thyroid nodules biologically. The Afirma Gene Expression Classifier (Afirma GEC; Veracyte) 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][16], Zheng et al [2015][17]); 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 outlines the testing algorithms for Afirma MTC and Afirma BRAF.

Table 1. Afirma MTC and Afirma BRAF testing Algorithms

Test 1	Test 1 Result	Reflex to Test 2
Thyroid nodule on fine needle aspirate	"Indeterminate"	Affirma MTC
Afirma GEC	"Malignant" or "Suspicious"	Affirma MTC
Afirma GEC	"Suspicious"	Affirma 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 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 of amount of DNA that is difficult to isolate from small FNA samples.[18]

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

Algorithmic Testing Using ThyGenX and ThyraMIR

The ThyGenX Thyroid Oncogene Panel (Interpace Diagnostics; 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 PTC and follicular carcinomas. ThyGenX has replaced the predicate miRInform Thyroid test that assesses for 17 validated gene alterations.

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

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

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (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 has chosen not to require any regulatory review of this test.

In 2013, the THxID™-BRAF kit (bioMérieux), an in vitro diagnostic device, was approved by the Food and Drug Administration (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® GEC	mRNA gene expression	167 genes	Benign/suspicious
Afirma® BRAF	mRNA gene expression	one gene	Negative/positive
Afirma® MTC	mRNA gene expression		Negative/positive
ThyroSeq v2, V3	Next-generation sequencing	60+ genes[b]	Specific gene variant/ translocation
ThyGenX™,[a]		eight genes	Specific gene variant/ translocation
TERT single-gene test	Unclear for commercially available test[c]	one gene	Specific gene variants
miRInform®,[a]	Multiplex PCR by sequence-specific probes	14 DNA variants, three RNA fusions	Specific gene variant/ translocation
ThyraMIR™	microRNA expression	10 microRNAs	Negative/positive
RosettaGX™ Reveal	microRNA expression	24 microRNAs	• Benign • Suspicious for malignancy • High risk for medullary carcinoma

FNA: fine needle aspirate; NGS: next-generation sequencing; PCR: polymerase chain reaction.
[a]The miRInform® test is the predicate test to ThyGenX™ and is not commercially available.
[b]Includes TERT.
[c] Available literature on TERT testing used PCR.

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.**

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

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75. National Government Services, Inc. (Primary Geographic Jurisdiction 06 & K - Illinois, Minnesota, Wisconsin, Connecticut, New York - Entire State, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont) Local Coverage Determination (LCD): Molecular Pathology Procedures (L35000), Revision Effective Date for services performed on or after 10/01/2018.