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FEP Medical Policy Manual

FEP 2.04.78 Molecular Markers in Fine Needle Aspiration of the Thyroid

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None

Molecular Markers in Fine Needle Aspiration of the Thyroid

Description

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.

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 v3 Next-Generation Sequencing panel (Sonic Healthcare) is an NGS panel of 112 genes. 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 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 available and stratify tissue from thyroid nodules biologically.

The Afirma Gene Expression Classifier (Afirma GEC; Veracyte) analyzed the expression of 142 different genes to determine patterns associated with benign findings on surgical biopsy. It was 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. In 2017, Veracyte migrated the Afirma GEC microarray analysis to a next-generation RNA sequencing platform and now markets the Afirma Gene Sequencing Classifier (Afirma GSC) which evaluates 10,196 genes with 1115 core genes.

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],[16] Zheng et al [2015][17]); they are not addressed in this review.

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 2 or more tests in a prespecified sequence, with a subsequent test automatically obtained depending on results of an earlier test.

Algorithmic Testing Using Afirma GEC With Afirma MTC and Afirma BRAF

In addition to Afirma GSC, 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 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"	Afirma MTC
Afirma GSC	"Malignant" or "suspicious"	Afirma MTC
Afirma GSC	"Suspicious"	Afirma BRAF

Afirma GSC: Afirma Gene Sequencing Classifier; Afirma MTC: Afirma medullary thyroid carcinoma

In a description of the Afirma BRAF test, the following have been proposed as benefits of the mRNA-based expression test for *BRAF* 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.[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- versus a total thyroidectomy or performance of central neck dissection.

Algorithmic Testing Using ThyGenX and ThyraMIR

The ThyGenX Thyroid Oncogene Panel (Interpace Diagnostics; testing is 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 PTC and follicular carcinomas. ThyGenX has replaced the predicate miR*Inform* 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.

OBJECTIVE

The objective of this evidence review is to evaluate whether testing for molecular markers in fine needle aspirates of the thyroid improves the net health outcome in individuals with thyroid nodule(s) with an indeterminate finding on the fine needle aspirate.

POLICY STATEMENT

For individuals who have thyroid nodules without strong clinical or radiologic findings suggestive of malignancy in whom surgical decision making would be affected by test results, the use of either of the following types of molecular marker testing or gene variant analysis in fine needle aspirates of thyroid nodules with indeterminate cytologic findings (ie, 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 **medically necessary**:

- Afirma Genomic Sequencing Classifier; or
- ThyroSeq.

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

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

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

POLICY GUIDELINES

In individuals 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 a single surgery.

Genetic Counseling

Experts recommend formal genetic counseling for individuals 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.

BENEFIT APPLICATION

Experimental or investigational procedures, treatments, drugs, or devices are not covered (See General Exclusion Section of brochure).

Screening (other than the preventive services listed in the brochure) is not covered. Please see Section 6 General exclusions.

Benefits are available for specialized diagnostic genetic testing when it is medically necessary to diagnose and/or manage a patient's existing medical condition. Benefits are not provided for genetic panels when some or all of the tests included in the panel are not covered, are experimental or investigational, or are not medically necessary.

FDA REGULATORY STATUS

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. Food and Drug Administration has chosen not to require any regulatory review of this test.

In 2013, the THxID™-BRAF kit (bioMrieux), an in vitro diagnostic device, was approved by the U.S. Food and Drug Administration 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 U.S. Food and Drug Administration. Table 2 provides a summary of commercially available molecular diagnostic tests for indeterminate thyroid pathology.

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

Test	Predicate	Methodology	Analyte(s)	Report
Afirma GSC	AfirmaGEC	mRNA gene expression	1115 genes	Benign/suspicious
Afirma BRAF		mRNA gene expression	1 gene	Negative/positive
Afirma MTC		mRNA gene expression		Negative/positive
ThyroSeq v3	ThyroSeq v2	Next-generation sequencing	112 genes	Specific gene variant/translocation
ThyGeNEXT	ThyGenX[a], miR*Inform*[a]	Next-generation sequencing	10 genes and 32 gene fusions	Specific gene variant/translocation

ThyraMIR™		microRNA expression	10 microRNAs	Negative/positive
RosettaGX™ Reveal		microRNA expression	24 microRNAs	• Benign • Suspicious for malignancy • High risk for medullary carcinoma

FNA: fine needle aspirate; GEC: Gene Expression Classifer; GSC: Gene Sequencing Classifier; mRNA: messenger RNA; MTC: medullary thyroid carcinoma; PCR: polymerase chain reaction.

[a] The miR*Inform* test is the predicate test to ThyGenX™ and is not commercially available.

RATIONALE

Summary of Evidence

For individuals with thyroid nodule(s) and indeterminate findings on fine needle aspiration (FNA) who receive FNA sample testing with molecular tests to rule out malignancy and to avoid surgical biopsy or resection, the evidence includes prospective clinical validity studies with the Afirma Gene Sequencing Classifier (GSC), a systematic review of prospective and retrospective clinical validity studies, a meta-analysis of real-world post validation data for the Afirma GSC platform with comparison to the validation study, and a chain of evidence to support clinical utility. Relevant outcomes are disease-specific survival, test accuracy and validity, morbid events, and resource utilization. A systematic review of 1 prospective and 6 retrospective trials demonstrated a high negative predictive value (NPV, 96%; 95% confidence interval [CI], 94% to 98%). In a multicenter validation study, the Afirma GSC was also reported to have a high NPV (96%; 95% CI, 90% to 99%). The meta-analysis of real-world Afirma GSC data indicated significantly higher NPV (as well as specificity and positive predictive value [PPV]) than in the validation study. These results are consistent with an earlier study on the Afirma GEC in the same study population and a randomized controlled trial of Afirma GSC in a similar population. In other multicenter and single-center studies, there is suggestive evidence that rates of malignancy are low in Afirma GSC or ThyroSeq v3 patients who are classified as benign or negative, with high NPVs (>90%) in a prospective trial with 31.8 months of post-testing imaging surveillance. The available evidence suggests that the decisions a physician makes regarding surgery are altered by Afirma GSC or ThyroSeq v3 results. A chain of evidence can be constructed to establish the potential for clinical utility with Afirma GSC and ThyroSeq v3 testing in cytologically indeterminate lesions, but evidence of improved outcomes must be demonstrated through at least 5 years of surveillance as recommended by the American College of Radiology. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule 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 for the initial resection. Prospective studies in additional populations are needed to validate these results. Although the presence of certain variants may predict more aggressive malignancies, the management changes that would occur as a result of identifying higher risk tumors, are not well-established. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and avoid surgical biopsy or to rule in malignancy for surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq test, a systematic review of retrospective and prospective studies, and 2 retrospective clinical validation studies that used a predicate test 17-variant panel (miR*Inform*) 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 (miR*Inform*) test and ThyraMIR had a sensitivity of 89%, and an NPV of 94%. A prospective clinical validation study of ThyroSeq v3 reported an NPV of 97% and PPV of 68%. Similarly, a systematic review including 3 prospective and 3 retrospective clinical validity studies reported an NPV of 92% and PPV of 70%. No studies were identified demonstrating the diagnostic characteristics of the marketed ThyGenX. No prospective studies were identified demonstrating evidence of direct outcome improvements. A chain of evidence for the ThyroSeq v3 test and combined ThyGenX and ThyraMIR testing would rely on establishing clinical validity. The evidence is insufficient to determine that the technology results in an improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

Guidelines or position statements will be considered for inclusion in 'Supplemental Information' if they were issued by, or jointly by, a US professional society, an international society with US representation, or National Institute for Health and Care Excellence (NICE). Priority will be given to guidelines that are informed by a systematic review, include strength of evidence ratings, and include a description of management of conflict of interest.

American Association of Clinical Endocrinologists et al

The American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazone Medici Endocrinologi (2016) updated their joint guidelines on molecular testing for cytologically indeterminate thyroid nodules, stating[59]:

- "Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the negative predictive values (NPVs) and positive predictive values (PPVs) for molecular testing."
- "Consider the detection of *BRAF* and *RET/PTC* and, possibly, *PAX8/PPARG* and *RAS* mutations if such detection is available."
- "*TERT* mutational analysis on FNA, when available, may improve the diagnostic sensitivity of molecular testing on cytologic samples."
- "Because of the insufficient evidence and the limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules."

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

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

American College of Radiology

The American College of Radiology (2017) Thyroid Imaging, Reporting, and Data System (TI-RADS) Committee published a white paper with expert consensus recommendations for FNA biopsy thresholds and imaging surveillance.[19,] Regarding timing of follow-up sonograms, the publication states: "We advocate timing on the basis of a nodule's ACR TI-RADS level, with additional sonograms for lesions that are more suspicious. For a TR5 lesion, we recommend scans every year for up to 5 years. For a TR4 lesion, scans should be done at 1, 2, 3, and 5 years. For a TR3 lesion, follow-up imaging may be performed at 1, 3, and 5 years. Imaging can stop at 5 years if there is no change in size, as stability over that time span reliably indicates that a nodule has a benign behavior. There is no published evidence to guide management of nodules that enlarge significantly but remain below the FNA size threshold for their ACR TI-RADS level at 5 years, but continued follow-up is probably warranted. If a nodule's ACR TI-RADS level increases on follow-up, the next sonogram should be done in 1 year, regardless of its initial level."

American Thyroid Association

The American Thyroid Association (2016) updated its guidelines on the management of thyroid nodules and differentiated thyroid cancer in adults.[60,] These guidelines made the following statements on molecular diagnostics in thyroid nodules that are atypia of undetermined significance or follicular lesion of undetermined significance on cytology and follicular neoplasm or suspicious for follicular neoplasm on cytology (see Table 3).

Table 3. Molecular Diagnostics in Thyroid Nodules on Cytology

Recommendation	SOR	QOE
AUS or FLUS		
"For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making."	Weak	Moderate
"If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference."	Strong	Low
FN or SFN		
"Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making."	Weak	Moderate

AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspirate; QOE: quality of evidence; SFN: suspicious for follicular neoplasm; SOR: strength of recommendation.

The guidelines also stated: "there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed."

National Comprehensive Cancer Network

National Comprehensive Cancer Network (v2.2023) guidelines on the treatment of thyroid cancer comment on the use of molecular diagnostics in thyroid cancer.[61,] For thyroid nodules evaluated with FNA, molecular diagnostics may be employed when lesions are suspicious for:

- Follicular or oncocytic neoplasms.
- Atypia of undetermined significance or follicular lesions of undetermined significance.

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

U.S. Preventive Services Task Force Recommendations

Not applicable.

Medicare National Coverage

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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POLICY HISTORY - THIS POLICY WAS APPROVED BY THE FEP® PHARMACY AND MEDICAL POLICY COMMITTEE ACCORDING TO THE HISTORY BELOW:

Date	Action	Description
September 2013	New policy	
September 2014	Replace policy	Policy updated with literature review. References 9, 15, 17-18, and 20-22 added. Policy statements remain unchanged.
September 2015	Replace policy	Policy title change. Policy updated with literature review. References 5-9, 15, 18, 20-24, and 28-29 added. Policy statement unchanged.
March 2017	Replace policy	Policy updated with literature review through March 30, 2016 and results of clinical input; references 9-10, 17-19, 23, 27, 33, 42-44, and 47-48 added. Medically necessary statement for the use of Afirma GEC in patients with indeterminate thyroid FNA added.
September 2018		Policy updated with literature review through April 9, 2018; references 4-10, 20, 26-30, 45-47, 65, and 69 added. Policy revised with updated genetics nomenclature. Policy statements revised to add medical necessity statements for ThyGenX, combined genetic variant analysis and microRNA gene expression classifier (ie, ThyGenX/ThyraMIR), Afirma BRAF after Afirma Gene Expression Classifier and Afirma MTC after Afirma Gene Expression Classifier. Policy statements revised to add investigational statement for TERT single-gene testing.
September 2019	Replace policy	Policy updated with literature review through May 14, 2019; references added and some references removed. "Afirma GEC" test replaced with new "Afirma GSC" test throughout the policy. Policy statements otherwise unchanged.
September 2020	Replace policy	Policy updated with literature review through June 12, 2020; references added. Edits made to the first policy statement; intent of statements unchanged. NCCN guideline updated.
September 2021	Replace policy	Policy updated with literature review through June 17, 2021; references added. Policy statements unchanged.
December 2022	Replace policy	Policy updated with literature review through June 14, 2022; references added. Minor editorial refinements to policy statements; intent unchanged.
December 2023	Replace policy	Policy updated with literature review through June 14, 2023; references added. Policy statements unchanged.