Sentara Health Plans

Molecular Markers in Fine Needle Aspirates of the Thyroid

Policy Number: AHS – M2108 – Molecular Markers in Fine Needle Aspirates of the Thyroid	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 12/01/2024	

I. Policy Description

Thyroid nodules are growths or enlargements on the thyroid gland. These nodules may be caused by several different disorders such as thyroiditis or cysts. However, these nodules may also be caused by thyroid cancer, which occurs in 4-6.5% of nodules. A biopsy is often performed to assess the histological components of this nodule, usually through a fine needle aspiration (FNA). A 23 to 27-gauge (commonly 25 gauge) needle is used, with or without local anesthesia. This technique can obtain an acceptable sample in 90-97% of solid nodules (Ross, 2022).

Molecular markers, such as genetic mutations or microRNA (miRNA) expression, may be used to help identify malignant nodules. Mutational analysis by sequencing or PCR can identify mutations, such as *BRAF*, *RAS*, *RET*/*PTC*, and *PAX8*/*PPARG*. Another tool is a gene expression classifier, which measures mRNA to determine the activity level of a number of genes and uses an algorithm to predict malignancy based on gene expression (Nikiforov et al., 2013).

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Applicable State and Federal Regulations of this policy document.

1) To assist in patient management decisions for individuals 18 years of age or older being evaluated for thyroid carcinoma, mutation analysis (e.g., *BRAF* V600, *RET/PTC, RAS, PAX8/PPARG*) and/or the use of a gene expression classifier in fine-needle aspirates (FNAs) of the thyroid **MEETS COVERAGE CRITERIA** when the FNA is cytologically characterized as **any** of the following:
   a) Bethesda-III (atypia of undetermined significance [AUS] or follicular lesion of undetermined significance [FLUS]).
   b) Bethesda-IV (follicular neoplasm [FN] or suspicious for follicular neoplasm [SFN]).

2) For individuals 18 years of age or older being evaluated for thyroid carcinoma, mutation analysis (e.g., *BRAF* V600, *RET/PTC, RAS, PAX8/PPARG*) and/or the use of a gene expression classifier in FNAs of the thyroid) **DOES NOT MEET COVERAGE CRITERIA** when the FNA is cytologically characterized as **any** of the following:
   a) Bethesda-I (nondiagnostic or unsatisfactory).
   b) Bethesda-II (benign).
   c) Bethesda-V (suspicious for malignancy).
   d) Bethesda-VI (malignant).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3) For individuals under 18 years of age, mutation analysis (e.g., *BRAF* V600, *RET/PTC, RAS, PAX8/PPARG*) or the use of a gene expression classifier in FNAs of the thyroid **DOES NOT MEET COVERAGE CRITERIA**.

4) MicroRNA profiling tests (e.g., ThyraMIR) in FNAs of the thyroid **DO NOT MEET COVERAGE CRITERIA**.

5) For all other situations not discussed above, mutation analysis (e.g., *BRAF* V600, *RET/PTC, RAS, PAX8/PPARG*) or the use of a gene expression classifier **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

III. Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
AAP	American Academy of Pediatrics
ACE	American College of Endocrinology
ALK	*Anaplastic lymphoma kinase* gene
ALS	*Argininosuccinate lyase* gene
AME	Associazione medici endocrinologi
ARID1A	*AT-rich interaction domain 1A* gene
ATA	American Thyroid Association
ATM	*ATM serine/threonine kinase* gene
AUS	Atypia of undetermined significance
BRAF	*B-Raf proto-oncogene* gene
CDKN2A	*Cyclin dependent kinase inhibitor 2A* gene

Term	Definition
CLIA/CAP	Clinical Laboratory Improvement Amendments/College of American Pathologists
CMS	Centers for Medicare and Medicaid
CTNNB1	*Catenin beta 1* gene
DNA	Deoxyribonucleic acid
ERBB2	*Erb-b2 receptor tyrosine kinase 2* gene
ERBB4	*Erb-b2 receptor tyrosine kinase 4* gene
ESMO	European Society for Medical Oncology
ETA	European Thyroid Association
FISH	Fluorescence in situ hybridization
FLUS	Follicular lesion of undetermined significance
FN	Follicular neoplasm
FNA	Fine needle aspiration
FNAs	Fine needle aspirates
GC	Genomic classifier
GEC	Gene expression classifier
GSC	Genomic sequencing classifier
HRAS	*HRAS proto-oncogene*
KRAS	*KRAS proto-oncogene*
LDTs	Laboratory-developed tests
MEN1	*Menin 1*
MET	*Met proto-oncogene, receptor tyrosine kinase* gene
MPTX	Thygenext expanded mutation panel
mRNA	Messenger ribonucleic acid
miRNA	Micro-ribonucleic acid
MSI	Microsatellite instability
MTC	Medullary thyroid cancer
NCCN	National comprehensive cancer network
NGS	Next-generation sequencing
NIFTP	Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
NKX2-1	NK2 homeobox 1
NPV	Negative predictive value
NTRK	*Neurotrophic tyrosine receptor kinase* gene
PAX8/PPARG	*Paired box gene 8/peroxisome proliferator-activated receptor gamma* gene
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PIK3CA	*Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* gene
PTEN	*Phosphatase and tensin homolog* gene
PPV	Positive predictive value
RAS	Rat sarcoma virus

Term	Definition
RET/PTC	*Rearranged during transfection)/papillary thyroid carcinoma type 1* gene
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SFN	Suspicious for follicular neoplasm
SMAD4	*SMAD family member 4* gene
SMO	*Smoothened, frizzled class receptor* gene
SRC	*Proto-oncogene tyrosine-protein kinase Src* gene
TBP	TATA-box binding protein
TERT	*Telomerase reverse transcriptase* gene
TMB	Tumor mutation burden
US	Ultrasound
USP33	Ubiquitin specific peptidase 33
XA	Xpression atlas

IV. Scientific Background

Fine needle aspiration (FNA) is a traditional diagnostic approach to differentiate malignant thyroid nodules that need surgery from benign nodules that do not require surgery. It offers definitive diagnosis in most cases; however, 20–30% of nodules yield an indeterminate cytologic diagnosis in which cancer cannot be ruled out, and such nodules can exhibit malignancy risk ranging from 12% to 33% (Jackson et al., 2020). This may lead to suboptimal management of these patients and can result in unnecessary resections and surgical interventions (Nikiforov et al., 2013). FNA results are normally categorized according to the National Cancer Institute into six categories. They are, in order of severity: nondiagnostic or unsatisfactory (Bethesda-I), benign (II), atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (III), follicular neoplasm (FN) or suspicious for follicular neoplasm (SFN) (IV), suspicious for malignancy (V), and malignant, which includes lymphomas and carcinomas (VI). A benign result is reported in 60-70% of FNAs. Nondiagnostic does not refer to an absence of cancer; rather it means that the sample provided was inadequate for a conclusive result and another sample must be provided. Categories III and IV are typically referred to as "indeterminate" and typically have a malignancy risk of as high as 40%. Patients with indeterminate nodules will frequently have a surgery to treat the issue; however, up to 95% of these nodules are ultimately evaluated as benign. Further testing must be done with indeterminate cases to categorize these lesions (Cibas & Ali, 2009; Douglas, 2023).

Molecular markers have been used to identify the true status of an indeterminate FNA. Assessing components of FNA aspirates, such as micro-RNA, mutational status of certain genes, or genomic sequencing could prove useful, especially as these components can be reliably detected during the FNA itself (Hodak & Rosenthal, 2013; Xing et al., 2013). Prior to the emergence of molecular markers as an indicator, a repeat FNA was often performed, ultimately leading to a surgery to remove the nodule. Molecular markers could thus reduce unnecessary surgeries as well as provide better risk stratification (Douglas, 2023).

Proprietary Testing

Commercially available panels of molecular markers utilizing FNA specimens from the thyroid include the following tests:

ThyGeNEXT and ThyraMIR

ThyGeNEXT (Interpace Diagnostics, Parsippany, NJ) is a specific oncogene, mutational panel that tests genetic alterations across 10 genes associated with papillary carcinoma and follicular carcinoma. ThyGeNEXT uses a next generation sequencing (NGS) platform to identify genetic alterations across those 10 genes, which are as follows: *ALK, BRAF, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TERT*. This test is primarily for category III or IV nodules. Recently, Interpace Diagnostics has developed a new molecular test, ThyraMIR. This test is based on microRNA analysis of the expression of 10 microRNAs. The manufacturer claims that this test can identify malignancy in nodules that otherwise evince no or "weak" mutation for ThyGeNEXT. According to Interpace Diagnostics, combined test performance has negative predictive value of 99%, positive predictive value of 96%, with 98% sensitivity and 98% specificity. The sensitivity and specificity for the ThyGeNEXT panel alone is 63% and 84%, respectively, for cases of indeterminant cytology (Interpace, 2023).

Lupo et al. (2020) featured a blinded multicenter study centered around the performance of the ThyGeNext expanded mutation panel (MPTX) and the expanded panel with the microRNA classifier ThyraMIR by comparing them to the histopathology diagnosis by three pathologists. The expanded mutation panel included *NTRK* and *ALS* fusions that have targeted therapies as well as proto-oncogenes *TERT* and *RET*, which are indicative of aggressive disease. The study found that the performance of the expanded mutation panel (ThyGeNext) alone unsatisfactory due to numerous false positives, 90% of which were attributed to individual *RAS* mutations primarily found in benign adenomas, with a few additional errors due to *TERT* mutations in benign disease. It was therefore reported that the expanded mutation panel of ThyGeNext alone demonstrated an NPV of 81% while the PPV was even less stellar, a mere 56% (Lupo et al., 2020). This contrasts with the reported high sensitivity for malignancy (95%) in negative mutation panel testing results and high (90%) specificity in nodules with Bethesda III and IV cytology, purportedly driving an increase in the NPV to 97% and the PPV to 75%, as aforementioned above. The researchers suggest that the increases in NPV and PPV from the inclusion of the microRNA classifier, therefore, possess great power and potential in ruling in and out the need for surgery for indeterminate thyroid nodule cytology, leading them to conclude that ancillary use of the three category MPTX approach can be leveraged to accurately inform the need for surgery in four out of five indeterminate nodules tested (Lupo et al., 2020).

The same optimism may be found in Jackson et al. (2020), where researchers aimed to better understand the incremental use of using expanded mutation panels, along with the integration of microRNA classifier testing to provide additional and more accurate diagnostic information. Using molecular results from two consecutive cohorts of patients totaling 12993 members, who had FNAs performed on thyroid nodules resulting in Bethesda Diagnostic categories III or IV cytology results, underwent either focused mutation panel testing (n=8619) alone or expanded mutation panel testing (n=4374), the latter of which included the microRNA classifier test ThyraMIR. The study found that 89% of patients who underwent the simple focused panel testing lacked detectable oncogenic mutations and fusions as compared to the 74% in the cohort who underwent the expanded panel testing ($P < 0.001$). Moreover, weak drivers were more frequently

identified in patients who underwent expanded (20%) compared with focused (9%) panel testing ($P < 0.001$), and strong drivers were likewise more frequent in patients who underwent expanded (6%) compared with focused (2%) panel testing ($P < 0.001$). Thus, the power of the expanded panel was not limited to detecting weak drivers, as 16% of those who underwent focused panel testing had strong drivers, while 24% of those who underwent expanded panel testing had strong drivers ($P < 0.001$). Inclusion of less common mutations in the expanded panel increased the detection of multiple coexisting mutations by 4%, which provides increased utility in identifying aggressive forms of thyroid cancer. However, the study concluded broadly that all oncogenic changes can contribute to neoplastic growth and progression, and therefore both strong and weak drivers should be considered clinically important. From the results of the study, the researchers contend that subsequent microRNA testing can help overcome uncertainty as to the presence of cancer, as approximately half of nodules with weak drivers had positive microRNA results consistent with a higher risk of malignancy, and 33% of those with positive microRNA results likewise had strong positive microRNA levels specific for malignancy that are prevalent in nodules with strong drivers (Jackson et al., 2020).

Ciarletto et al. (2021) used ThyraMIR to study if pairwise comparisons of differentially expressed miRNAs can identify medullary thyroid carcinoma in FNA. Differential pairwise analysis was performed on 10 miRNAs in 7557 specimens. Nine differential pairs were determined to have significant power to differentiate medullary thyroid carcinoma and non- medullary thyroid carcinoma samples. The test accuracy was 100%, “the assay correctly classified all MTC [medullary thyroid carcinoma] and non-MTC samples.” The authors conclude that “pairwise miRNA expression analysis of ThyraMIR results were found to accurately predict medullary thyroid carcinoma in thyroid FNA samples, including those with indeterminate FNA findings” (Ciarletto et al., 2021).

ThyroSeq v3

ThyroSeq is a test intended for assessment of thyroid nodules with undetermined cytology initially designed to target 12 cancer genes with 284 mutational hotspots. The latest version of this test, ThyroSeq v3, is based on NGS of DNA and RNA. This test detects 4 types of alterations; mutations, gene fusions, expression alterations, and copy number alterations. This test analyzes 112 genes, providing information on more than 12,000 mutation hotspots and more than 120 gene fusion types. First, the sample is ensured to have enough material to proceed (such as amount of thyroid follicular cells). Then, the NGS is performed and reviewed by a pathologist. Finally, the report is sent to the patient in about 2 weeks (ThyroSeq, 2023).

ThyroSeq test

In a multicenter study, this test was produced a negative predictive value of 97%, positive predictive value of 66%, with 94% sensitivity and 82% specificity in 257 cases of Bethesda-III and -IV nodules. The authors concluded that up to 61% of patients with indeterminate cytology could avoid diagnostic surgery through multigene genomic classifier testing (Steward et al., 2018). Other NGS-based molecular tests for thyroid nodules are available from other labs.

Nikiforova et al. (2018) evaluated the analytical performance of ThyroSeq v3 (intended to analyze 112 genes for alterations). A genomic classifier (GC) is used to differentiate malignant

nodules from benign. 238 tissue samples and 175 FNA samples were included in the cohort. ThyroSeq detected over 100 genetic alterations. The GC cutoff identified malignant nodules in the tissue samples from benign at 93.9% sensitivity, 89.4% specificity, and 92.1% accuracy. For the FNA sample, the GC sensitivity was 98.0%, the specificity was 81.8%, and the accuracy was 90.9% (Nikiforova et al., 2018).

Afirma Series

The Afirma Genomic Sequencing Classifier (GSC) is offered by Afirma. This test is intended to assess indeterminate nodules (Bethesda categories III and IV) and "conclusively rule out" surgery (Afirma, 2022a). This test is based on the Afirma Gene Expression Classifier (GEC), which measured the activity level of 167 genes. The GSC added several features to better classify nodules, such as classifiers for medullary thyroid cancer, parathyroid lesions, *BRAF V600E* mutations, and overall better discrimination of Hurthle cell neoplasms (Douglas, 2023). Wei et al note that the GSC is an "updated" version of the GEC, available since 2011. Wei et al also published a study discussing proposed advantages of the GSC over the GEC, namely its improved specificity (as a weakness of the GEC was its inability to identify true oncocytic lesions in the "suspicious" category). The authors compared the results of indeterminate FNA specimens (Bethesda categories III and IV) that were tested by Afirma GEC or GSC. 272 tests (194 GEC, 78 GSC) were evaluated. 221 samples were classified as AUS/FLUS and 51 were classified as FN/SFN. Out of the 194 samples tested with GEC, 88 were considered benign (45.4%) whereas 52 of the 78 GSC samples were considered benign (66.7%). In the AUS/FLUS category, 47.1% of cases were considered benign by GEC whereas 71.2% were considered benign by GSC (Wei et al., 2019).

A component of the Afirma GSC is the medullary thyroid cancer (MTC) component. This component includes 108 genes and is performed along with the primary GSC at no extra charge (Afirma, 2021a, 2021b). Randolph et al. performed a validation of this test using 211 samples (21 MTC cases, 190 controls), and all 211 samples were identified correctly. These sample results were confirmed by surgery (for positive cases) and pathology (for negative cases) (Randolph, 2017)

Another test is the Afirma Xpression Atlas. This test is a comprehensive panel encompassing 511 genes, 761 variants, and 130 fusion pairs. Genes such as *BRAF, RET*, and the *RAS* pathway of genes are included in this panel. This test is intended to inform surgical and therapeutic decisions for high-risk patients. Afirma lists three populations for this test; those who are "suspicious" per the Afirma GSC test, Bethesda category V, and Bethesda category VI. Angell et al validated this test. The XA was compared to the results of multiple other methodologies (such as whole-transcriptome RNA-seq, targeted DNA-seq, et al) and concordance was evaluated. 943 blinded FNA samples were used to compare the DNA and RNA detection and 695 blinded FNAs were used to compare the fusion detections. At the cutoff of 5% variant frequency, 74% of allele variants detected by traditional methods were also detected by the XA, with 88% detected by the XA at a 20% variant frequency cutoff. 82% of fusions detected by the targeted RNA fusion assay were detected by the XA. From an analytical validity standpoint, intra-plate reproducibility was found to be 89%-94%, and inter-plate reproducibility was found to be 86%-91% (Angell et al., 2019). More recently, Krane et al. (2020) compiled evidence for the analytical validity, clinical validity, and clinical utility of the Afirma series in an evidence-

based assessment. Analytical validation of the Afirma XA test using 69 variant-positive FNA samples demonstrated that there was high accuracy between the detection of variants (90%) and detection of fusion (94%) across two different laboratories. Clinical validation on the Afirma XA test's ability to detect genomic variants was measured against those of currently accepted methods of DNA and RNA sequencing using the aforementioned 943 FNA-blinded samples. However, it should be noted that 95% or more of the variants and fusions identified by Afirma XA can be identified simply through the reference DNA and RNA method. Moreover, some variants that were identified by DNA were absent or poorly expressed in RNA, and important promoter variants such as *TERT* are not identified by said test.

Jug et al. (2018) evaluated the performances of ThyroSeq and Afirma GEC within the context of ultrasonographic features and with the incorporation of the "noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) nomenclature." The ultrasonographic pattern evaluations were derived from the 2015 American Thyroid Association guidelines. A total of 304 cases were evaluated, with 119 resections. All cases that met criteria for NIFTP were considered high-risk by both tests. However, when these NIFTP cases were moved from the malignant to the non-malignant category, the positive predictive value of ThyroSeq dropped from 42.9% to 14.3% and Afirma's dropped from 30.1% to 25.3% (Jug et al., 2018).

Endo et al. (2019) compared Afirma's GEC to its Gene Sequencing Classifier (GSC), using cytologically indeterminate nodules. 343 GEC-tested nodules and 164 GSC-tested nodules were identified. The GSC was found to have a "statistically significant higher benign call rate (76.2% vs. 48.1%), PPV (60.0% vs. 33.3%), and specificity (94.3% vs. 61.4%)." The authors noted that the improvement was statistically significant for Bethesda III and IV nodules. The GSC benign call rate was significantly higher in Hürthle cell changes (88.8% vs 25.7%). 52.5% of indeterminate nodules went to surgery when using the GEC compared to only 17.6% when using the GSC (Endo et al., 2019). Vuong et al. (2021) completed a meta-analysis of seven studies comparing the clinical impact and diagnostic performance of Afirma's GEC and GSC. Similarly, this study showed that GSC had a higher benign cell rate, particularly in Hürthle cell predominated nodules, as well as a lower resection rate and higher risk of malignancy. The authors conclude that "the specificity (43.0% vs 25.1%; P = .003) and PPV (63.1% vs 41.6%; P = .004) of Afirma GSC were significantly improved while it still maintained a high sensitivity (94.3%) and a high NPV (90.0%)" compared to the GEC (Vuong et al., 2021).

RosettaGX Reveal

The RosettaGX Reveal test from Rosetta Genomics is a micro-RNA-based diagnostic test that evaluates indeterminate thyroid nodules. The test measures 24 sequences of miRNA through quantitative RT-PCR, as well as a medullary carcinoma marker (hsa-miR-375). Each sample is classified as benign or suspicious for malignancy.

The test has a claimed negative predictive value of 99%, a 98% sensitivity, and a 78% specificity from a sample of 150 in which three pathologists all agreed on the evaluation. Out of the 189 total samples, the negative predictive value was 91%, sensitivity was 85%, and specificity was 72%. The nodule sizes were >0.5 cm. The authors concluded that this test may be able to differentiate between malignant and benign from a previously evaluated indeterminate sample (Lithwick-Yanai et al., 2017). This test has been discontinued.

NeoGenomics

NeoGenomics offers a "NeoTYPE" thyroid profile, intended for evaluation of "fine needle aspirates of thyroid nodules that are indeterminate or suspicious on cytology." NeoGenomics states that FISH detects mutations and other gene rearrangements, and that *BRAF* mutation V600E is associated with poor prognosis of papillary thyroid carcinoma. The test measures the following genetic features: "*AKT1, ALK, ARID1A, ATM, BRAF, CDKN2A, CTNNB1, ERBB2, ERBB4, HRAS, KRAS, MEN1, MET*, Microsatellite Instability (MSI), *NF1, NF2, NRAS, PIK3CA, PTEN, RET, SMAD4, SMO, SRC, TERT* Promoter, *TP53, TSC1, TSC2,* Tumor Mutation Burden (TMB)", *MET* and *RET* by FISH, PD-L1, and Pan-TRK (NeoGenomics, 2022).

Clinical Utility and Validity

A study focusing on detection of *BRAF*, *RAS*, *RET*/*PTC*, and *PAX8*/*PPARg* mutations found that detection of any mutation resulted in a risk of histologic malignancy of 88%, 87%, and 95% for samples of Bethesda categories III, IV, and V, respectively. 967 samples were taken, and 87 mutations were found. The risk of cancer in mutation-negative samples was 6%, 14%, and 28%, respectively. Unfortunately, there was also a 14% false negative rate, limiting the usefulness of this panel (Douglas, 2023; Nikiforov et al., 2011).

Additional point mutations (including *TERT*, *TP53*, and others), as well as gene fusions that occur in thyroid cancer were found in another study using a next-generation sequencing (NGS) assay. 143 samples with a classification of follicular neoplasm or suspicious for follicular neoplasm were assessed, and the NGS panel "ThyroSeq v2" was used, which tests for point mutations in 13 genes and 42 types of gene fusions. 104 samples were found to be benign with the other 39 sample results being malignant. Overall, the NGS panel performed at a negative predictive value of 96%, a positive predictive value of 83%, 90% sensitivity, 93% specificity, and 92% accuracy for FN/SFN nodules (Nikiforov et al., 2014).

Another method that has been used is assessment of mRNA expression through a gene expression classifier. This is the basis of the Afirma genomic sequencing classifier, which identifies markers for features, such as medullary thyroid cancer, as well as distinguishes between Hürthle cell neoplasms from non-neoplastic Hürthle cell neoplasms. The second version of this test was assessed with 191 indeterminate samples. The sensitivity and specificity were 91% and 68%, respectively, and the negative and positive predictive values were 96% and 47%, respectively (Douglas, 2023; Patel et al., 2018). Afirma has produced a similar gene expression classifier "Xpression Atlas" that can be used to assess B-III to B-VI category neoplasms. Xpression Atlas evaluates a total of 761 gene variants and 130 fusion pairs (Afirma, 2022b; Douglas, 2023).

The above methods may be combined to better evaluate neoplasms, for instance, an miRNA gene expression classifier using both mutation analysis and miRNA expression. Labourier et al. (2015) evaluated a diagnostic algorithm that combined mutation analysis and miRNA expression to assess preoperative FNAs. This algorithm contained 17 validated gene alterations using the *miRInform* thyroid test and a 10-miRNA gene expression classifier. 109 samples of either AUS/FLUS or FN/SFN were evaluated with this combined algorithm, and this algorithm correctly identified 64% of malignant samples and 98% of benign ones. The sensitivity and specificity were 89% and 85%, respectively. For AUS/FLUS and FN/SFN, the negative

predictive value was 97% and 91%, and the positive predictive value for malignancy was 68% and 82%, respectively (Douglas, 2023; Labourier et al., 2015).

No single methodology has achieved clinical utility to reliably resolve all indeterminate cytology, and thus several professional organizations, including the American Association of Clinical Endocrinologists (AACE), the American Thyroid Association (ATA), and the National Comprehensive Cancer Network (NCCN), have published guidelines for the evaluation of thyroid nodules, all of which endorse a similar multistep strategy suggesting molecular markers can be of use when cytology is indeterminate, yet acknowledging its current limitations (Gharib et al., 2016; Haugen et al., 2016; NCCN, 2022).

Banizs et al. (2019) evaluated the utility of "combined mutation analysis and microRNA classification in reclassifying cancer risk of cytologically indeterminate thyroid nodules." A three-tiered microRNA threshold was determined based on nodules with known disease status, and an expected rate of malignancy calculated from mutation analysis and the microRNA approach was validated in an independent cohort of "atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm or suspicious for follicular neoplasm (FN/SFN) nodules with surgically derived outcomes." From there, 2685 patients were included in the intended analysis. 82% of these samples lacked mutations, with *BRAF, PIK3CA, PAX8/PPARg*, and *RET/PTC* mutations all comprising 2% or less. The maximum expected risk of malignancy in these nodules without mutation was 9% for AUS/FLUS nodules and 17% for FN/SFN nodules, but with positive microRNA status, these rates increased to 36% and 54% respectively. *RAS* mutations occurred in 15% of mutations, and the expected malignancy rates in nodules with *RAS* or *PAX8/PPARg* mutations was 49% for AUS/FLUS nodules and 65% for FN/SFN nodules. With positive microRNA status, these rates increased to 85% and 91%, respectively (Banizs & Silverman, 2019).

V. Guidelines and Recommendations

National Comprehensive Cancer Network

NCCN guidelines for thyroid carcinoma state that molecular diagnostic testing to detect individual mutations (such as *BRAF V600E* or *RET/PTC*) or pattern recognition approaches using molecular classifiers may be useful in the evaluating indeterminate FNA samples to assist in management decisions. NCCN states molecular diagnostics may be used to reclassify indeterminate lesions such as AUS/FLUS. Molecular markers should be interpreted within the context of each patient. The NCCN states that molecular testing may be considered to drive treatment decisions, and some mutations may have prognostic importance.

The NCCN further clarifies that, "The choice of the precise molecular test depends on the cytology and the clinical question being asked. Indeterminate groups include: 1) follicular or Hürthle cell neoplasms; and 2) AUS/FLUS." The NCCN panel recommends molecular diagnostic testing for evaluating FNA results that are suspicious for follicular cell neoplasms or AUS/FLUS (category 2A) and does not recommend testing on suspected Hürthle cell neoplasms as studies historically do not perform well on Hürthle neoplasms. However, the NCCN has acknowledged promising studies for assessing Hürthle neoplasms, with both the Afirma and ThyroSeq v3 tests providing better evaluations than their predecessors. The NCCN notes that

molecular diagnostic testing may include individual mutation analysis or a multigene assay such as a gene expression classifier. Molecular markers may also help with treatment decisions or with eligibility in clinical trials.

The NCCN further notes that active surveillance may be an option for patients whose molecular diagnostics demonstrate a risk of malignancy under 5% and that the predictive value of molecular diagnostics may be influenced by pre-test probability of disease based on various FNA cytology groups. The NCCN states that molecular diagnostic testing may be useful for follicular cell carcinomas and diagnosing NIFTP [noninvasive follicular thyroid neoplasm with papillary-like nuclear features], although current tests for assessing NIFTP have not been validated. Molecular testing is not recommended for diagnosing anaplastic thyroid carcinoma. Finally, the NCCN highlights that the diagnostic utility of molecular diagnostics in pediatric patients is still unclear because most of the published literature is on adult patients with thyroid nodules (NCCN, 2022).

American Thyroid Association

The 2015 ATA guidelines on the management of adult patients with thyroid nodules and differentiated thyroid cancer make the following recommendations on the use of molecular markers:

- "If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results." (Strong recommendation; low-quality evidence)
- "If intended for clinical use, molecular testing should be performed in Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP)-certified molecular laboratories, or the international equivalent, because reported quality assurance practices may be superior compared to other settings." (Strong recommendation; low-quality evidence)
- "For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making." (Weak recommendation; moderate-quality evidence)
- "Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making." (Weak recommendation; moderate-quality evidence)
- In general, only nodules >1 cm should be evaluated as they have a greater chance to become a clinically significant cancer. However, there are some cases where nodules <1 cm may be evaluated due to other clinical symptoms. The ATA states that "attempts to diagnose and treat all such small thyroid cancers in an effort to prevent exceedingly rare outcomes is deemed to cause more harm than good."

- "If the nodule is benign on cytology, further immediate diagnostic studies or treatment are not required."
- "Each nodule that is >1 cm carries an independent risk of malignancy and therefore multiple nodules may require FNA."

The guidelines also state that "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" (Haugen et al., 2016).

The ATA Guidelines Task Force on Pediatric Thyroid Cancer have developed unique guidelines for children and adolescents with thyroid tumors. They have presented 34 recommendations including recommendations on molecular markers testing and nodules. The ATA panel recommended the pediatric age to be limited to a patient that is ≤ 18 years of age to more accurately define the impact of the physiologic changes of growth and development on tumor behavior. These guidelines note that a size criterion is more difficult in children as age affects volume greatly and size of the nodule is not predictive of malignancy. Overall, studies focusing on molecular diagnostics in children have not been validated and so cannot be recommended at this time (Francis et al., 2015).

The ATA Guidelines for Management of Patients with Anaplastic Thyroid Cancer notes that FNA cytology can be an important diagnostic tool for ATC diagnosis but recommends a parallel core biopsy to obtain sufficient material for molecular testing and accurate diagnosis (ATA, 2021).

American Academy of Pediatrics

The AAP endorsed the guidelines of the American Thyroid Association Guidelines Task Force on Pediatric Thyroid Cancer (as presented in (Francis et al., 2015)) in a publication released in 2018 (AAP, 2018).

American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (Interpace) and Associazione Medici Endocrinology

These joint guidelines recommend the following:

- "Molecular testing should be considered to complement, not replace cytologic evaluation, and only if the results are expected to influence clinical management. As a general rule, molecular testing is not recommended in nodules with established benign or malignant cytologic characteristics."
- FNA is recommended for high ultrasound (US) risk lesions of ≥10 mm, intermediate risk lesions of >20 mm, and low risk lesions >20 mm and growing or with a risk history.
- FNA is not recommended for nodules that are functional on scintigraphy.
- Repeat FNA is recommended in benign nodules with suspicious clinical or US findings, a nondiagnostic initial FNA on a solid nodule, and nodules that become symptomatic or increase in volume by 50%. Pregnancy does not affect cytologic diagnostic criteria. Routine repeat FNA is generally not necessary.
- Nodules with a major diameter of <5 mm should be monitored instead of biopsied.

- Consider the detection of *BRAF* and *RET/PTC* and possibly *PAX8/PPARG* and *RAS* mutations if available.
- There is no stance on gene expression classifiers for indeterminate nodules, due to insufficient evidence and limited follow-up.
- There is also insufficient evidence to take any stance on mutation testing to guide surgery decisions, except on mutations with a PPV approaching 100% for papillary thyroid carcinoma such as BRAFV600E (Gharib et al., 2016).

European Thyroid Association

The ETA states that molecular testing for *BRAF, RET/PTC* and possibly *PAX8*, *PPARG,* and *RAS* mutations may be considered for cytologically indeterminate lesions. The search for molecular markers in B-II class lesions is not recommended, although one member of the panel did not agree with this non-recommendation. A GEC test cannot be recommended to exclude malignancy to replace diagnostic surgery or close surveillance, although one member did not agree with this non-recommendation. The targeted NGS approach is considered the most promising, with larger panels potentially becoming a rule-in and rule-out test if >95% negative predictive value can be reached. The ETA notes that molecular diagnostics may reduce completion thyroidectomies or other surgeries due to clearer assessments of an indeterminate lesion (Paschke et al., 2017).

European Society for Medical Oncology

ESMO remarks that preoperative FNA for cytology is "not required" for nodules 1 cm or smaller. ESMO states that FNA diagnosis "can be facilitated" by assessment of malignancy markers and molecular alterations. Specifically designed gene panels are "reportedly" useful for identifying malignancy with indeterminate samples (Filetti et al., 2019).

VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81479	Unlisted molecular pathology procedure
81546	Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious) Proprietary test: Afirma® Genomic SequencingClassifier Lab/Manufacturer: Veracyte, Inc
81599	Unlisted multianalyte assay with algorithmic analysis
0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy Proprietary test: ThyraMIR Lab/Manufacturer: Interpace Diagnostics
0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy") Proprietary test: Thyroseq Genomic Classifier Lab/Manufacturer: CBLPath, Inc/University of Pittsburgh Medical Center
0204U	Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected Proprietary test: Afirma Xpression Atlas Lab/Manufacturer: Veracyte, Inc
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage

CPT	Code Description
	Proprietary test: ThyGeNEXT® Thyroid Oncogene Panel Lab/Manufacturer: Interpace Diagnostics
0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high) Proprietary test: ThyroSeq® CRC Lab/Manufacturer: CBLPath, Inc/University of Pittsburgh Medical Center

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

AAP. (2018). Management Guidelines for Children With Thyroid Nodules and Differentiated Thyroid Cancer. *Pediatrics*, *142*(6). https://doi.org/10.1542/peds.2018-3063

Afirma. (2021a). Case Study: Genomic Analysis Identifies Medullary Thyroid Carcinoma. https://www.afirma.com/physicians/practice-tools/newsletter/case-study-genomic-analysis-identifies-medullary-thyroid-carcinoma/

Afirma. (2021b). Clinical Validation of the Afirma Genomic Sequencing Classifier for Medullary Thyroid Cancer. https://www.afirma.com/physicians/ata-2017/qa-6/

Afirma. (2022a). https://www.afirma.com/physicians/why-afirma/#a1

Afirma. (2022b). *NEW Afirma GSC + Xpression Atlas*. https://www.afirma.com/physicians/practice-resources/#a6

Angell, T. E., Wirth, L. J., Cabanillas, M. E., Shindo, M. L., Cibas, E. S., Babiarz, J. E., Hao, Y., Kim, S. Y., Walsh, P. S., Huang, J., Kloos, R. T., Kennedy, G. C., & Waguespack, S. G. (2019). Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples [10.3389/fendo.2019.00612]. *Frontiers in Endocrinology*, *10*, 612. https://www.frontiersin.org/article/10.3389/fendo.2019.00612

ATA. (2021). 2021 American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer. *Thyroid*, *31*(3), 337-386. https://doi.org/10.1089/thy.2020.0944

Banizs, A. B., & Silverman, J. F. (2019). The utility of combined mutation analysis and microRNA classification in reclassifying cancer risk of cytologically indeterminate thyroid nodules. *Diagn Cytopathol*, *47*(4), 268-274. https://doi.org/10.1002/dc.24087

Ciarletto, A. M., Narick, C., Malchoff, C. D., Massoll, N. A., Labourier, E., Haugh, K., Mireskandari, A., Finkelstein, S. D., & Kumar, G. (2021). Analytical and clinical validation of pairwise microRNA expression analysis to identify medullary thyroid cancer in thyroid fine-needle aspiration samples. *Cancer Cytopathol*, *129*(3), 239-249. https://doi.org/10.1002/cncy.22365

Cibas, E. S., & Ali, S. Z. (2009). The Bethesda System for Reporting Thyroid Cytopathology. *American Journal of Clinical Pathology*, *132*(5), 658-665. https://doi.org/10.1309/AJCPPHLWMI3JV4LA

Douglas, R. (2023, 01/24/2023). *Evaluation and management of thyroid nodules with indeterminate cytology*. https://www.uptodate.com/contents/evaluation-and-management-of-thyroid-nodules-with-indeterminate-cytology

Endo, M., Nabhan, F., Porter, K., Roll, K., Shirley, L. A., Azaryan, I., Tonkovich, D., Perlick, J., Ryan, L. E., Khawaja, R., Meng, S., Phay, J. E., Ringel, M. D., & Sipos, J. A. (2019). Afirma Gene Sequencing Classifier Compared with Gene Expression Classifier in Indeterminate Thyroid Nodules. *Thyroid*, *29*(8), 1115-1124. https://doi.org/10.1089/thy.2018.0733

Filetti, S., Durante, C., Hartl, D., Leboulleux, S., Locati, L. D., Newbold, K., Papotti, M. G., Berruti, A., & Committee, t. E. G. (2019). Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology*, *30*(12), 1856-1883. https://doi.org/10.1093/annonc/mdz400 %J Annals of Oncology

Francis, G. L., Waguespack, S. G., Bauer, A. J., Angelos, P., Benvenga, S., Cerutti, J. M., Dinauer, C. A., Hamilton, J., Hay, I. D., Luster, M., Parisi, M. T., Rachmiel, M., Thompson, G. B., & Yamashita, S. (2015). Management Guidelines for Children with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, *25*(7), 716-759. https://doi.org/10.1089/thy.2014.0460

Gharib, H., Papini, E., Garber, J. R., Duick, D. S., Harrell, R. M., Hegedus, L., Paschke, R., Valcavi, R., Vitti, P., & Nodules, A. A. A. T. F. o. T. (2016). American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the Diagnosis and Management of Thyroid Nodules--2016 Update. *Endocr Pract*, *22*(5), 622-639. https://doi.org/10.4158/EP161208.GL

Haugen, B. R., Alexander, E. K., Bible, K. C., Doherty, G. M., Mandel, S. J., Nikiforov, Y. E., Pacini, F., Randolph, G. W., Sawka, A. M., Schlumberger, M., Schuff, K. G., Sherman, S. I., Sosa, J. A., Steward, D. L., Tuttle, R. M., & Wartofsky, L. (2016). 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, *26*(1), 1-133. https://doi.org/10.1089/thy.2015.0020

Hodak, S. P., & Rosenthal, D. S. (2013). Information for clinicians: commercially available molecular diagnosis testing in the evaluation of thyroid nodule fine-needle aspiration specimens. *Thyroid*, *23*(2), 131-134. https://doi.org/10.1089/thy.2012.0320

Interpace. (2023). https://thygenext-thyramir.com/

Jackson, S., Kumar, G., Banizs, A. B., Toney, N., Silverman, J. F., Narick, C. M., & Finkelstein, S. D. (2020). Incremental utility of expanded mutation panel when used in combination with microRNA classification in indeterminate thyroid nodules. *Diagnostic Cytopathology*, *48*(1), 43-52. https://doi.org/https://doi.org/10.1002/dc.24328

Jug, R. C., Datto, M. B., & Jiang, X. S. (2018). Molecular testing for indeterminate thyroid nodules: Performance of the Afirma gene expression classifier and ThyroSeq panel. *Cancer Cytopathol*, *126*(7), 471-480. https://doi.org/10.1002/cncy.21993

Krane, J. F., Cibas, E. S., Endo, M., Marqusee, E., Hu, M. I., Nasr, C. E., Waguespack, S. G., Wirth, L. J., & Kloos, R. T. (2020). The Afirma Xpression Atlas for thyroid nodules and thyroid cancer metastases: Insights to inform clinical decision-making from a fine-needle aspiration sample. *Cancer Cytopathology*, *128*(7), 452-459. https://doi.org/https://doi.org/10.1002/cncy.22300

Labourier, E., Shifrin, A., Busseniers, A. E., Lupo, M. A., Manganelli, M. L., Andruss, B., Wylie, D., & Beaudenon-Huibregtse, S. (2015). Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology. *J Clin Endocrinol Metab*, *100*(7), 2743-2750. https://doi.org/10.1210/jc.2015-1158

Lithwick-Yanai, G., Dromi, N., Shtabsky, A., Morgenstern, S., Strenov, Y., Feinmesser, M., Kravtsov, V., Leon, M. E., Hajdúch, M., Ali, S. Z., VandenBussche, C. J., Zhang, X., Leider-Trejo, L., Zubkov, A., Vorobyov, S., Kushnir, M., Goren, Y., Tabak, S., Kadosh, E., . . . Meiri, E. (2017). Multicentre validation of a microRNA-based assay for diagnosing indeterminate thyroid nodules utilising fine needle aspirate smears. *J Clin Pathol*, *70*(6), 500-507. https://doi.org/10.1136/jclinpath-2016-204089

Lupo, M. A., Walts, A. E., Sistrunk, J. W., Giordano, T. J., Sadow, P. M., Massoll, N., Campbell, R., Jackson, S. A., Toney, N., Narick, C. M., Kumar, G., Mireskandari, A., Finkelstein, S. D., & Bose, S. (2020). Multiplatform molecular test performance in indeterminate thyroid nodules. *Diagnostic Cytopathology*, *48*(12), 1254-1264. https://doi.org/https://doi.org/10.1002/dc.24564

NCCN. (2022, 11/01/2022). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Thyroid Carcinoma*. https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf

NeoGenomics. (2022). NeoTYPE® Thyroid Profile. https://neogenomics.com/test-menu/neotype-thyroid-profile

Nikiforov, Y. E., Carty, S. E., Chiosea, S. I., Coyne, C., Duvvuri, U., Ferris, R. L., Gooding, W. E., Hodak, S. P., LeBeau, S. O., Ohori, N. P., Seethala, R. R., Tublin, M. E., Yip, L., & Nikiforova, M. N. (2014). Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. *Cancer*, *120*(23), 3627-3634. https://doi.org/10.1002/cncr.29038

Nikiforov, Y. E., Ohori, N. P., Hodak, S. P., Carty, S. E., LeBeau, S. O., Ferris, R. L., Yip, L., Seethala, R. R., Tublin, M. E., Stang, M. T., Coyne, C., Johnson, J. T., Stewart, A. F., & Nikiforova, M. N. (2011). Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. *J Clin Endocrinol Metab*, *96*(11), 3390-3397. https://doi.org/10.1210/jc.2011-1469

Nikiforov, Y. E., Yip, L., & Nikiforova, M. N. (2013). New strategies in diagnosing cancer in thyroid nodules: impact of molecular markers. *Clin Cancer Res*, *19*(9), 2283-2288. https://doi.org/10.1158/1078-0432.ccr-12-1253

Nikiforova, M. N., Mercurio, S., Wald, A. I., Barbi de Moura, M., Callenberg, K., Santana-Santos, L., Gooding, W. E., Yip, L., Ferris, R. L., & Nikiforov, Y. E. (2018). Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. *Cancer*, *124*(8), 1682-1690. https://doi.org/10.1002/cncr.31245

Paschke, R., Cantara, S., Crescenzi, A., Jarzab, B., Musholt, T. J., & Sobrinho Simoes, M. (2017). European Thyroid Association Guidelines regarding Thyroid Nodule Molecular Fine-Needle Aspiration Cytology Diagnostics. *Eur Thyroid J*, *6*(3), 115-129. https://doi.org/10.1159/000468519

Patel, K. N., Angell, T. E., Babiarz, J., & et al. (2018). Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. *JAMA Surgery*, *153*(9), 817-824. https://doi.org/10.1001/jamasurg.2018.1153

Randolph, G., Angell, Trevor, Barbiarz, Joshua, Barth, Neil. (2017). ABSTRACT: Clinical Validation of the Afirma Genomic Sequencing Classifier for Medullary Thyroid Cancer. 87th Annual Meeting of the American Thyroid Association,

Ross, D. (2022, 06/07/2022). *Diagnostic approach to and treatment of thyroid nodules*. https://www.uptodate.com/contents/diagnostic-approach-to-and-treatment-of-thyroid-nodules

Steward, D. L., Carty, S. E., Sippel, R. S., & et al. (2018). Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: A prospective blinded multicenter study. *JAMA Oncology*. https://doi.org/10.1001/jamaoncol.2018.4616

ThyroSeq. (2023). https://thyroseq.com/physicians/test-details/test-description

Vuong, H. G., Nguyen, T. P. X., Hassell, L. A., & Jung, C. K. (2021). Diagnostic performances of the Afirma Gene Sequencing Classifier in comparison with the Gene Expression Classifier: A meta-analysis. *Cancer Cytopathol*, *129*(3), 182-189. https://doi.org/10.1002/cncy.22332

Wei, S., Veloski, C., Sharda, P., & Ehya, H. (2019). Performance of the Afirma genomic sequencing classifier versus gene expression classifier: An institutional experience. *Cancer Cytopathology*, *127*(11), 720-724. https://doi.org/10.1002/cncy.22188

Xing, M., Haugen, B. R., & Schlumberger, M. (2013). Progress in molecular-based management of differentiated thyroid cancer. *Lancet*, *381*(9871), 1058-1069. https://doi.org/10.1016/s0140-6736(13)60109-9

IX. Review/Revision History

Effective Date	Summary
12/01/2024	Initial Policy Implementation