Thyroid Cancer Molecular Panel

Indications for Ordering

Patients with a clinically suspicious thyroid nodule and indeterminate fine-needle aspirate (FNA) cytology may benefit from this test when considered in conjunction with imaging and cytology results

Test Description

BRAF, HRAS, KRAS, and *NRAS* genes
- Polymerase chain reaction/pyrosequencing

RET-CCDC6, *RET-NCOA4*, and *PAX8-PPARG* translocations
- Reverse transcription polymerase chain reaction (RT-PCR)
  - Tumor RNA is isolated from formalin-fixed, paraffin-embedded (FFPE) tissue blocks, liquid cytology, or FNA specimens and reverse transcribed into cDNA
  - cDNA is amplified by PCR using oligonucleotide primers for *RET-CCDC6 (PTC1)*, *RET-NCOA4 (PTC3)*, and *PAX8-PPARG* fusions
  - Amplification of a cDNA control target within the *MRPL19* gene is performed to confirm that RNA quality is acceptable for testing

Tests to Consider

Primary test

Thyroid Translocation and Mutation Panel 2012755
- Detects mutations in *BRAF, HRAS, KRAS,* and *NRAS* genes
- Detects *RET-CCDC6(PTC1)*, *RET-NCOA4(PTC3)*, and *PAX8-PPARG* translocations
- More cost effective than ordering multiple single-gene assays

Related tests

BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498
- Detects activating *BRAF* mutation (codon 600) associated with thyroid cancer
- Use prior to initiation of BRAF V600E inhibitor therapy

BRAF V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921
- Determines *BRAF* V600E mutation status in patients with solid tumors to select candidates for targeted therapy with kinase inhibitors (BRAF and/or MEK)
- Monitors response to therapy and disease progression in patients carrying *BRAF* V600E mutation

HRAS Mutation Detection by Pyrosequencing 2012175
- Detects *HRAS* mutations (codons 12, 13, 61) associated with thyroid cancer

KRAS Mutation Detection 0040248
- Detects *KRAS* mutations (codons 12, 13, 61) associated with thyroid cancer
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies

NRAS Mutation Detection by Pyrosequencing 2003123
- Detects *NRAS* mutations (codons 12, 13, 61) associated with thyroid cancer
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies

PAX8-PPARG Translocations Detection by PCR 2012603
- Detects *PAX8-PPARG* gene fusions associated with thyroid cancer

RET-CCDC6 and *RET-NCOA4* (*RET-PTC1* and *RET-PTC3*) Translocations by PCR 2012605
- Detects *RET-CCDC6* and *RET-NCOA4* gene fusions associated with thyroid cancer

RET Gene Rearrangements by FISH 2012654
- Detects *RET* gene rearrangements in solid tumors
  - May be useful for detection of other *RET* gene translocations in thyroid cancer that are not detected by test 2012605
  - Does not identify the translocation partner or variant

Disease Overview

Incidence

Thyroid cancer is the most common endocrine tumor
- ~13.5/100,000 individuals

Diagnostic issues

- Usually presents as a thyroid nodule
  - Most nodules are benign
- FNA – most used for evaluation of thyroid nodules
- >90% of thyroid cancers carry certain gene mutations
  - Mutation detection may aid in diagnosis/prognostication
  - Most commonly reported
    - *BRAF, RAS* gene mutations
    - *RET-PTC, PAX8-PPARG* translocations

Genetics

Genes – *BRAF, HRAS, KRAS, NRAS* (see tables for more information)

Fusion transcripts (translocations)

- *PAX8* gene fusion partner – *PPARG*
- *RET* gene fusion partners – *CCDC6(PTC1), NCOA4(PTC3)*

Test Interpretation

Sensitivity/specificity – see tables

Results – see tables

Limitations – see tables

References

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Gene Mutations				
Gene Name	**Mutation**	**Sensitivity/Specificity**	**Results**	**Limitations**
BRAF Function Kinase-encoding gene in the RAS/RAF/MAPK pathway	Majority of oncogenic mutations are in codon 600	Clinical sensitivity – activating *BRAF* mutations detected in 40-45% of papillary thyroid carcinomas (PTC)	Mutations are highly suggestive of PTC in FNA specimens with indeterminate cytology	Oncogenic mutations other than codon 600 will not be detected
HRAS Function GTPase-encoding gene in the RAS/RAF/MAPK pathway	Majority of oncogenic mutations are in codon 61 (57%) Most remaining activating mutations are in codons 12, 13 (43%)	Clinical sensitivity – activating *HRAS* mutations detected in ~4% of thyroid cancers	Isolated mutation is not diagnostic of malignancy, but suggestive for neoplasm in a thyroid nodule	Oncogenic mutations other than codons 12, 13, and 61 will not be detected
KRAS Function GTPase-encoding gene in the RAS/RAF/MAPK pathway	Majority of oncogenic mutations are in codons 12, 13 (>80%) Most remaining activating mutations are in codons 61, 146	Clinical sensitivity – activating *KRAS* mutations detected in ~3% of thyroid cancers Mutation can be detected in thyroid adenoma	Presence of mutation is suggestive of neoplasm in an indeterminate thyroid nodule	Oncogenic mutations other than codons 12, 13, and 61 will not be detected
NRAS Function GTPase-encoding gene in the RAS/RAF/MAPK pathway	Majority of oncogenic mutations are in codon 61	Clinical sensitivity – activating NRAS mutations detected in ~6% of thyroid cancers Mutation can be detected in thyroid adenoma	Presence of mutation is suggestive of neoplasm in an indeterminate thyroid nodule	Oncogenic mutations other than codons 12, 13, and 61 will not be detected

Gene Translocations				
Gene Name	**Translocation Partner**	**Sensitivity/Specificity**	**Results**	**Gene**
RET Function Encodes a receptor tyrosine kinase protein that activates the MAPK pathway	***RET-CCDC6(PTC1)*** Fusion of *RET* and *PTC1* genes	Clinical sensitivity – detected in 10-20% of adult PTCs Most common in PTC – relatively specific for this subtype	Translocations are highly suggestive of PTC in FNA specimens with indeterminate cytology	Translocations other than *RET-CCDC6(PTC1)* and *RET-NCOA4(PTC3)* will not be detected
	RET-NCOA4(PTC3) Fusion of *RET* and *PTC3* genes	Clinical sensitivity – detected in 10-20% of adult PTCs More common in solid variant of PTC		
PAX8 Function Encodes protein that is essential for formation of thyroxine-producing follicular cells	***PAX8-PPARG*** Fusion of *PAX8* and *PPARG* genes	Clinical sensitivity – detected in 30-40% of conventional-type follicular thyroid cancers Also occurs in follicular variant of PTC and in follicular adenoma	Presence of translocation is suggestive of neoplasm in FNA specimens with indeterminate cytology	Translocations other than *PAX8-PPARG* will not be detected