Test Definition: RASFP

RAS/RAF Panel, Tumor

Overview

Useful For

Identifying tumors that may respond to targeted therapies by assessing multiple gene targets simultaneously

Identifying mutations that may help determine prognosis forpatients withsolid tumors

Identifying specific mutations within genes known to be associated with response or resistance to specific cancer therapies

Genetics Test Information

This extended RAS/RAF panel test uses targeted next-generation sequencing to evaluate for somatic mutations within the *BRAF* (exons 11 and 15), *HRAS* (exons 2 and 3), *NRAS* (exons 2, 3, 4), and *KRAS* (exons 2, 3, 4) genes. This includes, but is not limited to, the testing of somatic mutations in *KRAS* codons 12, 13, 59, 61, 117, 146; *NRAS* codons 12, 13, 59, 61,146; *HRAS* codons 12, 13, 61; and *BRAF* codons 594, 596, 600. See Targeted Gene Regions Interrogated by *RAS*/*RAF* Gene Panel in Special Instructions for details regarding the targeted gene regions identified by this test.

Of note, this test is performed to evaluate for somatic mutations within tumor samples. This test does not assess for germline alterations within the genes listed.

Highlights

This test provides evaluation of *BRAF* (including V600), *HRAS*, *NRAS* (includes codons 12, 13, 59, 61, and 146), and *KRAS* (includes codons 12, 13, 59, 61, 117, and 146) genes for somatic mutations.

Additional Tests

Test ID	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No	Yes

Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

Special Instructions

- Targeted Gene Regions Interrogated by RAS/RAF Gene Panel
- Tissue Requirements for Solid Tumor Next-Generation Sequencing

Method Name

Polymerase Chain Reaction (PCR)-Based Next Generation Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Necessary Information

Pathology report (final or preliminary) at minimum containing the following information must accompany specimen in order for testing to be performed:

1. Patient name

2. Block number-must be on all blocks, slides and paperwork (can be handwritten on the paperwork)

3. Tissue collection date

4. Source of the tissue

Specimen Required

This assay requires at least 20% tumor nuclei.

-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue144 mm(2)

-Minimum amount of tumor area: tissue 36 mm(2).

-These amounts are cumulative over up to 10 unstained slides and must have adequate percent tumor nuclei.

-Tissue fixation: 10% neutral buffered formalin, not decalcified

-For specimen preparation guidance, see Tissue Requirement for Solid Tumor Next-Generation Sequencing in Special Instructions. In this document, the sizes are given as 4mm x 4mm x 10 slides as preferred: approximate/equivalent to 144 mm(2) and the minimum as 3mm x 1mm x 10 slides: approximate/equivalent to 36mm(2).

Preferred:

Specimen Type: Tissue block

Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block with acceptable amount of tumor tissue.

Acceptable:

Specimen Type: Tissue slide

Slides: 1 stained and 10 unstained

Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 10 unstained, nonbaked slides with 5-micron thick sections of the tumor tissue.

Note: The total amount of required tumor nuclei can be obtained by scraping up to 10 slides from the same block.

Specimen Type: Cytology slide (direct smears or ThinPrep)

Slides: 1 to 3 slides

Collection Instructions: Submit 1 to 3 slides stained and cover slipped with a preferred total of 5000 nucleated cells or a minimum of at least 3000 nucleated cells.

Note: Glass coverslips are preferred; plastic coverslips are acceptable but will result in longer turnaround times.

Additional Information: Cytology slides will not be returned.

Forms

If not ordering electronically, complete, print, and send an Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

Other	Specimens that have been decalcified (all methods) Specimens that have not been formalin-fixed, paraffin-embedded

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Frozen		
	Refrigerated		

Clinical and Interpretive

Clinical Information

Targeted cancer therapies are defined as antibody or small molecule drugs that block the growth and spread of cancer by interfering with specific cell molecules involved in tumor growth and progression. Multiple targeted therapies have been approved by the FDA for treatment of specific cancers. Molecular genetic profiling is often needed to identify targets amenable to targeted therapies and to minimize treatment costs and therapy-associated risks.

Next generation sequencing has recently emerged as an accurate, cost-effective method to identify mutations across numerous genes known to be associated with response or resistance to specific targeted therapies. The results of this test can be useful for assessing prognosis and guiding treatment of individuals with solid tumors. These data can also be used to help determine clinical trial eligibility for patients with mutations in genes not amenable to current FDA-approved targeted therapies.

The epidermal growth factor receptor (EGFR) gene product is activated by the binding of specific ligands (epiregulin and amphiregulin), resulting in activation of the RAS/MAPK pathway. Activation of this pathway induces a signaling cascade ultimately regulating a number of cellular processes including cell proliferation. Dysregulation of the RAS/MAPK pathway is a key factor in tumor progression. Targeted therapies directed to EGFR, which inhibit activation of the RAS/MAPK pathway, have demonstrated some success (increased progression-free and overall survival) in patients with colorectal cancer.

Assessment for *BRAF* mutations has clinical utility in that it is a predictor of response to antimutant BRAF therapy. BRAF is a member of the mitogen-activated protein/extracellular signal-regulated (MAP/ERK) kinase pathway, which plays a role in cell proliferation and differentiation. Dysregulation of this pathway is a key factor in tumor progression. Targeted therapies directed to components of this pathway have demonstrated some success with increases both in progression-free and overall survival in patients with certain tumors. Effectiveness of these therapies, however, depends in part on the mutation status of the pathway components.

See Targeted Gene Regions Interrogated by *RAS*/*RAF* Gene Panel in Special Instructions for details regarding the targeted gene regions identified by this test.

Reference Values

An interpretive report will be provided.

Interpretation

An interpretive report will be provided.

Cautions

This test cannot differentiate between somatic and germline alterations. Additional testing may be necessary to clarify the significance of results if there is a potential hereditary risk.

DNA variants of uncertain significance may be identified.

A negative (wild-type) result does not rule out the presence of a mutation that may be present but below the limits of detection of this assay (approximately 5%-10%).

This test does not detect large single or multiexon deletions or duplications or genomic copy number variants.

Rare polymorphisms may be present that could lead to false-negative or false-positive results. Test results should be interpreted in the context of clinical findings, tumor sampling, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for updated interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Clinical Reference

1. Targeted Cancer Therapies. National Cancer Institute Fact Sheet. Updated 12/05/2012. Accessed December 2013, Available at www.cancer.gov/cancertopics/factsheet/Therapy/targeted

2. Vogelstein B, Papadopoulos N, Velculescu VE, et al: Cancer genome landscapes. Science 2013;339:1546-1558

3. Beadling C, Neff TL, Heinrich MC, et al: Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. J Mol Diagn 2013;15:171-176

4. Anderson S, Bloom KJ, Vallera DU, et al: Multisite analytic performance studies of a real-time polymerase chain reaction assay for the detection of BRAF V600E mutations in formalin-fixed paraffin-embedded tissue specimens of malignant melanoma. Arch Pathol Lab Med 2012 Feb;136:1-7

5. Di Nicolantonio F, Martini M, Molinari F, et al: Wild-type BRAF is required for response to Panitumumab or Cetuximab in metastatic colorectal cancer. J Clin Oncol 2008;26:5705-5712

6. Flaherty KT, Puzanov I, Kim KB, et al: Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010;363(9):809-819

7. Ladanyi M, Pao W: Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol 2008;21

Suppl 2:S16-S22

8. Lievre A, Bachet JB, Le Corre D, et al: KRAS mutation status is predictive of response to Cetuximab therapy in colorectal cancer. Cancer Res 2006;66(8):3992-3995

Performance

Method Description

Next-generation sequencing is performed to test for the presence of a mutation in targeted regions of the following 4 genes: *BRAF, HRAS, NRAS, KRAS.*(Unpublished Mayo method)

See Targeted Gene Regions Interrogated by *RAS*/*RAF* Gene Panel in Special Instructions for details regarding the targeted gene regions identified by this test.

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday; Varies

Analytic Time

12 days

Maximum Laboratory Time

20 days

Specimen Retention Time

Unused portions of blocks will be returned. Unused slides are stored indefinitely.

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

RAS/RAF Targeted Gene Panel by Next Generation Sequencing, Tumor

81210-*BRAF* (v-raf murine sarcoma viarl oncogene humolog B1) (eg, colon cancer), gene analysis, V600E variant

81275-*KRAS* (v-Ki-ras2 Kirsten rat sarcoma viral oncogene) (eg, carcinoma) gene analysis, variants in codons 12

and 13

81403-*HRAS* (v-Ha-ras Harvey rat sarcoma viral oncogene homolog) (eg, Costello syndrome), exon 2 sequence

81311-*NRAS* (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)

Slide Review

88381-Microdissection, manual

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
RASFP	RAS/RAF Panel, Tumor	In Process

Result ID	Test Result Name	Result LOINC Value
36725	Result Summary	50397-9
36726	Result	82939-0
36727	Interpretation	69047-9
36728	Additional Information	48767-8
36729	Specimen	31208-2
36730	Source	31208-2
36731	Tissue ID	80398-1
36732	Released By	18771-6