MAYO CLINIC LABORATORIES

Test Definition: NTRKM

NTRK Genes Mutation Analysis,
Next-Generation Sequencing, Tumor

Overview

Useful For

Identifying *NTRK* mutations that may predict resistance to Trk inhibitors

Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *NTRK1, NTRK2,* and *NTRK3* genes. See Targeted Genes and Methodology Details for NTRK Genes Mutation Analysis for details regarding the targeted gene regions evaluated by this test.

This test is performed to evaluate for somatic mutations within solid tumor samples. This test **does not assess** for germline alterations within the genes listed.

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No, (Bill Only)	Yes

Testing Algorithm

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

Special Instructions

- Tissue Requirements for Solid Tumor Next-Generation Sequencing
- Targeted Genes and Methodology Details for NTRK Genes Mutation Analysis

Highlights

This test evaluates formalin-fixed, paraffin-embedded tumor or cytology slides from patients with advanced solid tumors for gene mutations in the *NTRK1*, *NTRK2*, and *NTRK3* genes. Current data suggests that identifying a *NTRK* gene mutation may predict resistance to first generation Trk inhibitors.

Method Name

Sequence Capture Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Multiple oncology (cancer) gene panels are available. For more information see Oncology Somatic NGS Testing Guide.

Necessary Information

A pathology report (final or preliminary), at minimum containing the following information, **must accompany specimen 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: tissue 216 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 this document, the sizes are given as 4 mm x 4 mm x 10 slides as preferred: approximate/equivalent to 144 mm(2) and the minimum as 3 mm x 1 mm x 10 slides: approximate/equivalent to 36 mm(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.
Additional Information: Unused unstained slides will not be returned.

Specimen Type: Cytology slide (direct smears or ThinPrep)
Slides: 1 to 3 Slides
Collection Instructions: Submit 1 to 3 slides stained and coverslipped 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 a Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume
See Specimen Required

Reject Due To

Specimens that have been decalcified (all methods) Specimens that have not been formalin-fixed, paraffin-embedded, except for cytology slides Extracted nucleic acid (DNA/RNA)	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information
The *NTRK1*, *NTRK2*, and *NTRK3* genes encode the tropomyosin receptor kinases TrkA, TrkB, and TrkC, respectively. Fusions of the *NTRK* genes with a variety of 5' (upstream) partner genes upregulate Trk kinase activity and contribute to tumorigenesis. *NTRK* gene fusions have been reported in diverse tumor types. Numerous US Food and Drug Administration approved pan-Trk inhibitors have been developed for the treatment of tumors with *NTRK* gene fusions. However, resistance to Trk inhibition can occur through the development of *NTRK* gene mutations. This test can be used to identify *NTRK* resistance mutations to aid in the management of these patients. Second generation Trk inhibitors have been developed to overcome resistance to the first-generation inhibitors.

Reference Values
An interpretive report will be provided.

Interpretation
The interpretation of molecular biomarker analysis includes an overview of the results and the associated diagnostic,

prognostic, and therapeutic implications.

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 result does not rule out the presence of a variant that may be present but below the limits of detection of this assay. The analytical sensitivity of this assay for sequence reportable alterations is 5% mutant allele frequency with a minimum coverage of 500X in a sample with 20% or more tumor content.

Point mutations and small insertion/deletion mutations will be detected in the *TERT* promoter region only. This test does not detect large single or multi-exon deletions or duplications or genomic copy number variants in *TERT* promoter region.

Variant allele frequency (VAF) is the percentage of sequencing reads supporting a specific variant divided by the total sequencing reads at that position. In somatic testing, VAF should be interpreted in the context of several factors including, but not limited to, tumor purity/heterogeneity/copy number status (ploidy, gains/losses, loss of heterozygosity) and sequencing artifact/misalignment.(1,2)

Rare polymorphisms may be present that could lead to false-negative or false-positive results.

The presence or absence of a variant may not be predictive of response to therapy in all patients.
Test results should be interpreted in the context of clinical, tumor sampling, histopathological, and other laboratory data. If results obtained do not match other clinical or laboratory findings, contact the laboratory for discussion. Misinterpretation of results may occur if the information provided is inaccurate and/or incomplete.

Reliable results are dependent on adequate specimen collection and processing. This test has been validated on cytology slides and formalin-fixed, paraffin-embedded tissues; other types of fixatives are discouraged. Improper treatment of tissues, such as decalcification, may cause polymerase chain reaction failure.

Supportive Data

Performance Characteristics:
The limit of detection for calling a somatic variant (single nucleotide variants [SNV] and deletions-insertions [delins, formerly indel]) is 5% variant allele frequency (VAF) and having at least 500x deduplicated coverage.

Verification studies demonstrated concordance between this test and the reference method for detection of SNV and delins is 98.5% (673/683) and 98.4% (122/124) of variants, respectively. Concordance for the detection of delins was 99.0% (100/101) in variants 1 to 10 base pairs (bp) in size, 93.3% (14/15) in variants 11 to 50 bp in size, and 100% (8/8) in variants over 50 bp in size.

To ensure accuracy, this test will be performed on cases that are estimated by a pathologist to have at least 20% tumor cells.

Clinical Reference

1. Strom SP. Current practices and guidelines for clinical next-generation sequencing oncology testing. Cancer Biol Med. 2016;13(1):3-11. doi:10.28092/j.issn.2095-3941.2016.0004
2. Spurr L, Li M, Alomran N, et al. Systematic pan-cancer analysis of somatic allele frequency. Sci Rep. 2018;8(1):7735. Published 2018 May 16. doi:10.1038/s41598-018-25462-0
3. WHO Classification of Tumours Editorial Board: Central nervous system tumours. 5th ed. World Health Organization; 2021. WHO Classification of Tumours. Vol 6.
4. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci USA. 2013;110(15):6021-6026
5. Koelsche C, Sahm F, Capper D, et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. Acta Neuropathol. 2013;126(6):907-915
6. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med. 2015;372(26):2499-2508
7. Cancer Genome Atlas Research Network, Brat DJ, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372(26):2481-2498
8. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LAl. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957-959
9. Schulze K, Imbeaud S,Letouze E, et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet. 2015;47(5):505-511

Performance

Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *NTRK1, NTRK2,* and *NTRK3* genes.(Unpublished Mayo method)

A pathology review and macro dissection to enrich for tumor cells is performed prior to slide scraping.

PDF Report

No

Day(s) Performed

Monday through Friday

Report Available

12 to 20 days

Specimen Retention Time

FFPE tissue block: Unused portions of blocks will be returned 10 to 14 days after testing is complete; FFPE tissue/cytology slides: Unused slides are stored indefinitely; Digital images are obtained and stored for all slides used in testing.

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

88381-Microdissection, manual
81479

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
NTRKM	NTRK Genes Mutation Analysis, Tumor	105596-1

Result ID	Test Result Name	Result LOINC® Value
619704	Result	82939-0
619705	Interpretation	69047-9
619706	Additional Information	48767-8
619707	Specimen	31208-2
619708	Tissue ID	80398-1
619709	Method	85069-3
619710	Disclaimer	62364-5
619711	Released By	18771-6