MAYO CLINIC LABORATORIES

Test Definition: IDTRT

IDH1, IDH2, and TERT Mutation Analysis, Next-Generation Sequencing, Tumor

Overview

Useful For

Identifying specific mutations within the *IDH1 and IDH2* genes and the *TERT* promoter to assist in tumor diagnosis/classification

Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *IDH1* and *IDH2* genes and the *TERT* promoter. See Targeted Genes and Methodology Details for IDH1/2 and TERT 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 IDH1/2 and TERT Mutation Analysis

Method Name

Sequence Capture and Targeted 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 Hematology and Oncology Test Selection 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: Submit 3, if available, or 2 of the following specimens
Acceptable: Submit **at least one** of the following specimens.

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

Specimen Type: Tissue slide
Slides: 1 Hematoxylin and eosin-stained and 10 unstained
Collection Instructions:
Submit the followings slides:
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:
1. Hematoxylin and eosin-stained and unstained slides will not be returned.
2. Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

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 total of 5000 nucleated cells (preferred) or at least 3000 nucleated cells (minimum).

Note: Glass coverslips are preferred; plastic coverslips are acceptable but will result in longer turnaround times.
Additional Information:
1. Cytology slides will not be returned. An image of the slides will be stored per regulatory requirements.
2. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

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

Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information
The *IDH1* and *IDH2* (IDH) genes encode enzymes involved in cellular glucose metabolism. Mutations in the IDH genes primarily involve codons R132 in *IDH1* and R140 and R172 in *IDH2*, and lead to the neomorphic ability to generate oncometabolite R(-)-2-hydroxyglutarate, which contributes to tumorigenesis.

TERT gene encodes the catalytic subunit of telomerase, an enzyme complex that regulates telomere length. Mutations in the *TERT* promoter primarily involve the mutational hotspot positions c.-124 (also known as C228) and c.-146 (also known as C250) and increase telomerase activity allowing tumor cells to overcome cellular senescence.

IDH and *TERT* promoter mutations are essential diagnostic molecular biomarkers for diffuse gliomas, a group of central

nervous system (CNS) tumors. Testing for both biomarkers is often diagnostically necessary. This test simultaneously assesses for somatic mutations involving the IDH and *TERT* promoter genes. IDH and *TERT* promoter mutations are also molecular biomarkers for a variety of non-CNS tumors but usually do not co-occur and would be best evaluated by stand-alone IDH and *TERT* promoter tests.

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 below the limits of detection of this assay. In a specimen with 20% or more tumor content, the analytical sensitivity of this assay for sequence reportable alterations is 5% mutant allele frequency with a minimum coverage of 500X.

Point mutations and small deletion-insertion mutations (delins) will only be detected in the *IDH1* and *IDH2* genes and the *TERT* promoter. This test may detect single exon deletions but does not detect multi-exon deletions, duplications, larger-scale genomic copy number variants, copy neutral loss of heterozygosity, or epigenetic modifications such as promoter methylation. Delins of 1,000 bp or less are detectable with at least 50 or more supporting reads.

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 alterations (ie, polymorphisms) may be present that could lead to false-negative or false-positive results.

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 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.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

Supportive Data

Performance Characteristics:
The limit of detection for calling a somatic variant (single nucleotide variants [SNV] and deletions/insertions [delins]) 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, Verhaak RGW, et al: Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372(26):2481-2498
8. Yan H, Parsons DW, Jin G, et al: IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360(8):765-773

Performance

Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in the *TERT* promoter and all coding regions of the *IDH1* and *IDH2* genes. See Targeted Genes and Methodology Details for IDH1/2 and TERT Mutation Analysis for details regarding the targeted gene regions identified by this test.(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
Tissue blocks: Unused portions of blocks will be returned; Tissue slides: Hematoxylin and eosin-stained and unstained slides will not be returned. Unused slides are stored for at least 5 years; Extracted DNA: 3 months

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
0481U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
IDTRT	IDH1/2 and TERT Mutation Analysis	105587-0

Result ID	Test Result Name	Result LOINC® Value
619650	Result	82939-0
619651	Interpretation	69047-9
619652	Additional Information	48767-8
619653	Specimen	31208-2
619654	Tissue ID	80398-1
619655	Method	85069-3
619656	Disclaimer	62364-5

619657	Released By	18771-6