Test Definition: TFH

FH Mutation Analysis, Next-Generation Sequencing, Tumor

Overview

Useful For

Identifying specific mutations within the *FH* gene to assist in tumor diagnosis/classification, including renal cell carcinoma, uterine/cutaneous leiomyoma, and pheochromocytoma/paraganglioma

Genetics Test Information

This test uses targeted next-generation sequencing to evaluate for somatic mutations within the *FH* gene. See Targeted Genes and Methodology Details for FH 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** germline alterations within the *FH* gene.

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 FH Mutation Analysis

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 Hematology, Oncology, and Hereditary 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 Requirements 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: Hematoxylin and eosin-stained and 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 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: Cytology slides will not be returned. An image of the slides will be stored per regulatory requirements.

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

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Disease-causing alterations of the fumarate hydratase (*FH*) gene have been implicated in renal cell carcinoma, uterine/cutaneous leiomyoma, and pheochromocytoma/paraganglioma. While these tumors can occur in the sporadic setting, germline alterations of the *FH* gene have been associated with renal cell carcinoma, and uterine/cutaneous leiomyoma in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome.

The 5th edition of the World Health Organization Classification of Tumours recognizes fumarate hydratase-deficient renal cell carcinoma as a molecularly defined entity.(1) This single gene assay, performed using formalin-fixed paraffin-embedded tissue or cytology material, is therefore helpful in documenting an underlying disease-causing alteration of the *FH* gene and is diagnostically significant. It is important to note that this assay does not distinguish between germline alterations and somatic mutations.

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 mutations 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 (delins) mutations will be detected in *FH* gene only. 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 1000 base pairs 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.(2,3)

Rare genetic 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.

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. WHO Classification of Tumours Editorial Board, eds. Urinary and male genital tumours. 5th ed. World Health Organization; 2022. WHO Classification of Tumours. Vol 8
2. 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
3. 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
4. Trpkov K, Hes O, Williamson SR, et al: New developments in existing WHO entities and evolving molecular concepts: The Genitourinary Pathology Society (GUPS) update on renal neoplasia. Mod Pathol. 2021;34(7):1392-1424
5. Forde C, Lim DHK, Alwan Y, et al. Hereditary leiomyomatosis and renal cell cancer: Clinical, molecular, and screening

features in a cohort of 185 affected individuals. Eur Urol Oncol. 2020;3(6):764-772
6. Gupta S, Swanson AA, Chen YB, et al. Incidence of succinate dehydrogenase and fumarate hydratase-deficient renal cell carcinoma based on immunohistochemical screening with SDHA/SDHB and FH/2SC. Hum Pathol. 2019;91:114-122
7. Carlo MI, Hakimi AA, Stewart GD, et al. Familial kidney cancer: Implications of new syndromes and molecular insights. Eur Urol. 2019;76(6):754-764
8. Nguyen KA, Syed JS, Espenschied CR, et al. Advances in the diagnosis of hereditary kidney cancer: Initial results of a multigene panel test. Cancer. 2017;123(22):4363-4371

Performance

Method Description

Next-generation sequencing is performed to evaluate the presence of a mutation in all coding regions of the *FH* gene. See Targeted Genes and Methodology Details for FH Mutation Analysis for details regarding the targeted gene regions identified by this test.(Unpublished Mayo method)

A pathology review and macro dissection to enrich 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
81405

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
TFH	FH Mutation Analysis, Tumor	49872-5

Result ID	Test Result Name	Result LOINC® Value
619659	Result	82939-0
619660	Interpretation	69047-9
619661	Additional Information	48767-8
619662	Specimen	31208-2
619663	Tissue ID	80398-1
619664	Method	85069-3
619665	Disclaimer	62364-5
619666	Released By	18771-6