MAYO CLINIC LABORATORIES

Test Definition: MCSTP

MayoComplete Solid Tumor Panel, Next-Generation Sequencing, Tumor

Overview

Useful For

Assisting in tumor profiling for diagnosis, predicting prognosis, and identifying targeted therapies for the treatment and management of patients with solid tumors

Identifying somatic alterations including single nucleotide variants, small deletions/insertions, gene amplifications, fusions, and splice variants in genes known to be associated with the tumorigenesis of solid tumors

Assessment of microsatellite instability and tumor mutational burden status

Genetics Test Information

This test uses targeted next-generation sequencing to estimate tumor mutational burden, determine microsatellite instability status, and identify somatic sequence variants, gene amplifications, fusions, and specific transcript variants in solid tumors. This panel includes a DNA subpanel for the detection of sequence alterations in 515 genes and amplification of 59 genes as well as an RNA subpanel for the detection of fusions involving 55 genes and specific splice variants involving *EGFR*, *AR*, and *MET*. See Genes Interrogated by MayoComplete Solid Tumor Panel for details regarding genes interrogated by this test.

Note: This test is performed to evaluate for somatic (ie, tumor-specific) alterations within the genes listed. Although germline (ie, inherited) alterations may be detected, this test cannot distinguish between germline and somatic alterations with absolute certainty. Follow-up germline testing using whole blood can be performed for confirmation of suspected clinically relevant germline alterations. Germline testing should be performed along with genetic counselling.

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 to ensure specimen adequacy.

Special Instructions

- Genes Interrogated by MayoComplete Solid Tumor Panel
- MayoComplete Solid Tumor Panel DNA Panel Excluded DNA Regions

Highlights

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, Oncology, and Hereditary Test Selection Guide.

Necessary Information

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. However, 40% tumor is preferred.
-Preferred amount of tumor area: 720 mm(2) tissue on up to 20 unstained slides
-Minimum amount of tumor area: 192 mm(2) tissue on up to 20 unstained slides
-Tissue fixation: 10% neutral buffered formalin, not decalcified
-For this test, at least 6mm x 6mm areas on 20 unstained slides is preferred: this is approximately equivalent to 720 mm(2). The minimum acceptable area is 3.1mm x 3.1mm on 20 unstained slides: approximately equivalent to 192 mm(2).

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

Acceptable:
Specimen Type: Tissue slide
Slides: 1 stained and 20 unstained
Collection Instructions: Submit 1 hematoxylin and eosin (H and E) stained slide and 20 unstained, nonbaked 5-micron thick sections
Note: The total amount of required tumor can be obtained by scraping up to 20 slides from the same block.

Specimen Type: Cytology slides (direct smears or ThinPrep)

Slides: 2 to 6 slides
Collection Instructions: Submit 2 to 6 stained and cover slipped slides with a preferred total of 10,000 nucleated cells or a minimum of at least 6000 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. An image of the slides will be stored per regulatory requirements.

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 Bone marrow in EDTA	Reject

Specimen Stability Information

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

Clinical & 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 US Food and Drug Administration for treatment of solid tumor malignancies. Molecular genetic profiling is often needed to identify targets amenable to targeted therapies and to minimize treatment costs and therapy-associated risks. Tumor mutational burden and microsatellite instability status are increasingly important biomarkers for determining effective immunotherapeutic treatment options for patients with solid tumors.(1,2)

In addition to providing therapeutic insight, molecular profiling of tumors often provides prognostic and diagnostic information. Next-generation sequencing is an accurate, cost-effective method to identify variants across numerous genes known to be associated with response or resistance to specific targeted therapies. This test is a single assay that uses formalin-fixed paraffin-embedded tissue or cytology specimens to assess for Tier I and Tier II variants in 515 genes known to be associated with solid tumors.(3)

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
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.

To ensure accuracy, this test will be performed on cases that are estimated by a pathologist to have 20% or more tumor cells, however, for optimal performance of this assay, a tumor purity of 40% is recommended.

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

This test does not detect large structural variants, copy number changes, or insertions, deletions, or duplications greater than approximately 20 base pairs in size.

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

A negative (ie, wildtype) result does not rule out the presence of an alteration that may be present but below the limits of detection of this assay.

The presence or absence of a variant or rearrangement may not be predictive of response to therapy in all patients.

A list of genomic regions in the DNA panel that have insufficient coverage to reliably detect single nucleotide variants and small deletions/insertions are listed in MayoComplete Solid Tumor Panel DNA Panel Excluded DNA Regions.

Supportive Data
Performance Characteristics

Verification studies demonstrated concordance between this test and the reference method for detection of single

nucleotide variants (SNV) and deletions-insertions (delins) in 98.8% (503/509) and 98.6% (294/298) of variants, respectively. Detection accuracy of delins was 99.3% (277/279) in variants 1 to 10 base pairs (bp) in size, 93.3% (14/15) in variants 11 to 20 bp in size, and 75.0% (3/4) in variants greater than 20 bp in size.

The limit of detection for calling a somatic variant (SNV and small delins) is 2% variant allele frequency (VAF) having at least 150X median exon coverage depth.

Gene amplification is identified at a 2.2x fold change based on the normalized read depth over the copy number variant (CNV) target divided by the normalized read depth of the inferred diploid genome. Gene amplification detection is most accurate at 40% or more tumor cells. For gene amplifications, overall sensitivity was 98.7% (81/82), specificity was 92.3% (24/26), and concordance was 95.3% (105/108) during verification studies.

Of the 130 microsatellite sites available for evaluation in this assay, at least 20% of sites are required to be unstable to classify the case as MSI-High. Microsatellite instability (MSI) evaluation is most accurate at a tumor purity of 40% or more, although, highly unstable tumors may be detectable at 20% tumor. During verification studies, 100% concordance was observed between this test and orthogonal methods used to detect MSI status.

Tumor mutational burden (TMB) was measured as mutations per megabase (Mb) for regions with greater than 50X coverage. When TMB scores were classified as TMB-Low (<10 mut/Mb) or TMB-High (> or =10 mut/Mb), 83% (50/60) concordance was achieved between this test and orthogonal assays detecting TMB status. Of the 10 samples with conflicting qualitative classification (ie, TMB-Low or TMB-High), the TMB quantitative values were near the 10 mut/Mb cutoff (3.9-11.8 mut/Mb). TMB values are most accurate at greater than or equal to 40% tumor purity.

Fusions are detected with the presence of 3 or more supporting reads passing pipeline filters and splice variants with 10 or more supporting reads. For fusions and splice variants, overall sensitivity was 98.0% (151/154), specificity was 94.8% (91/96), and overall concordance was 96.8% (242/250). Fusion and splice variant detection are most accurate at greater than or equal to 20% tumor purity.

Table 1. Analytical Sensitivity

Variant type	Threshold for positivity	Recommended tumor purity
SNV	> or =2% VAF > or =150X median exon coverage	> or =20%
DELIN	> or =2% VAF, < or =20 bp	> or =20%
Gene amplification	> or =2.2X fold change	> or =40%
MSI status	> or =20% sites unstable= MSI-H	> or =40%
TMB status	> or =10 variants per megabase= TMB-H	> or =40%
Fusion	> or =3 supporting reads	> or =20%
Splice variant	> or =10 supporting reads	> or =20%

Clinical Reference

1. Subbiah V, Solit DB, Chan TA, Kurzrock R: The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) > or =10: a decision centered on empowering patients and their physicians. Ann Oncol. 2020 Sep;31(9):1115-1118. doi: 10.1016/j.annonc.2020.07.002
2. Marcus L, Lemery SJ, Keegan P, Pazdur R: FDA Approval Summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. Clin Cancer Res. 2019 Jul 1;25(13):3753-3758. doi: 10.1158/1078-0432.CCR-18-4070
3. Li MM, Datto M, Duncavage EJ, et al: Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diag. 2017 Jan;19(1):4-23. doi: 10.1016/j.jmoldx.2016.10.002

Performance

Method Description
Next-generation sequencing is performed to estimate tumor mutational burden and microsatellite instability status, somatic sequence variants, gene amplifications, fusions, and specific transcript variants in solid tumors. This test detects single nucleotide variants and small insertions and deletion within 515 genes, amplification of 59 genes, gene fusions involving 55 genes, and splice variants involving *EGFR*, *AR*, and *MET*.(Instruction manual: TruSight Oncology 500 High-Throughput. Illumina; 11/2020)

See Genes Interrogated by MayoComplete Solid Tumor Panel for details regarding genes interrogated by this test.

PDF Report
Supplemental

Day(s) Performed
Varies

Report Available
14 to 21 days

Specimen Retention Time
FFPE tissue: Unused portions of FPPE blocks will be returned. Unused, unstained slides: 5 years; Stained slides: Indefinitely.

Performing Laboratory Location
Rochester

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

81459
88381-Microdissection, manual

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MCSTP	MayoComplete Solid Tumor Panel	73977-1

Result ID	Test Result Name	Result LOINC® Value
610425	Result	82939-0
610426	Interpretation	69047-9
610427	Additional Information	48767-8
610428	Clinical Trials	82786-5
610429	Variants of Uncertain Significance	93367-1
610430	Specimen	31208-2
610431	Tissue ID	80398-1
610432	Method	85069-3
610433	Disclaimer	62364-5
610434	Released By	18771-6