MAYO CLINIC LABORATORIES

Test Definition: MCLNM

MayoComplete Lung Cancer Mutations, Next-Generation Sequencing, Tumor

Overview

Useful For

Diagnosis and management of patients with lung cancer

Assessing microsatellite instability

Genetics Test Information

This test uses targeted next-generation sequencing to determine microsatellite instability status and to evaluate for somatic mutations within the *ALK, BRAF, EGFR, ERBB2, HRAS, KRAS, MDM2, MET, NRAS, RET, ROS1,* and *STK11* genes. See Targeted Genes and Methodology Details for MayoComplete Lung Cancer Mutations for details regarding the targeted gene regions evaluated by this test.

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

This test identifies activating exon 14 skipping mutations in *MET*

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 MayoComplete Lung Cancer Mutations

Highlights

This test evaluates formalin-fixed, paraffin-embedded tumor or cytology slides from patients with lung cancer for gene mutations to identify candidates for targeted therapy.

Microsatellite instability (MSI) status is determined (microsatellite stable, MSI-High) as part of this test and is often clinically actionable for determining the efficacy of immunotherapy in solid tumors.

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 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:
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

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 specific cancers. Molecular genetic profiling is often needed to identify targets amenable to targeted therapies and to minimize treatment costs and therapy-associated risks. Microsatellite instability status is an increasingly important biomarker for determining effective immunotherapeutic treatment options for patients with solid tumors.

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.

This test is a single assay that uses formalin-fixed paraffin-embedded tissue to assess for common mutations in the following genes known to be associated with lung cancer: *ALK, BRAF, EGFR, ERBB2, HRAS, KRAS, MDM2, MET, NRAS, RET, ROS1,* and *STK11*. The results of this test can be useful for assessing prognosis and guiding treatment of individuals with lung cancer.

Current data suggests that:
-The efficacy of EGFR-targeted therapies in patients with non-small cell lung cancer is limited to tumors with mutations in the *EGFR* gene
-Metastatic non-small cell lung cancer with *BRAF V600E* mutations may be sensitive to targeted therapy
-Metastatic non-small cell lung cancer with *KRAS G12C* mutations may be sensitive to targeted therapy
-Advanced or metastatic non-small cell lung cancer with *MET* exon 14 skipping mutations may be sensitive to MET 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 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 deletion-insertion mutations (delins) will be detected in the *ALK, BRAF, EGFR, ERBB2, HRAS, KRAS, MDM2, MET, NRAS, RET, ROS1,* and *STK11* genes 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.(1,2)

Rare alterations (ie, 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 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 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 99.7% (699/701) and 96.6% (226/234) of variants, respectively. Concordance for the detection of delins was 98.9% (186/188) in variants 1 to10 base pair (bp) in size, 95.8% (23/24) in variants 11 to 50 bp in size, and 88.9% (8/9) in variants 51 to 200 bp in size.

Microsatellite instability (MSI) evaluation is accurate at a tumor purity of at least 10% for colorectal tumors and 20% for other tumor types. During verification studies, 98% (200/204) concordance for MSI status was observed between this test and the reference method.

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. U.S. Food and Drug Administration (FDA). Table of Pharmacogenomic Biomarkers in Drug Labeling. FDA; Updated August 11, 2022, Accessed July 31, 2023. Available at: www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling
4. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer. 2007;7(3):169-181
5. Mok TS. Personalized medicine in lung cancer: What we need to know. Nat Rev Clin Oncol. 2011;8(11):661-668
6. Cheng L, Alexander RE, Maclennan GT, et al, Molecular pathology of lung cancer: key to personalized medicine. Mod Path. 2012;25(3):347-369
7. Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. 2006;118(2):257-262
8. Gao G, Ren S, Li A, et al. Epidermal growth factor receptor-tyrosine kinase inhibitor therapy is effective as first-line treatment of advanced non-small-cell lung cancer with mutated EGFR: A meta-analysis from six phase III randomized controlled trials. Int J Cancer. 2012;131(5):E822-829. doi:10.1002/ijc.27396
9. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol. 2005;23(25):5900-5909
10. Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET Inhibitors. Cancer Discov. 2015;5(8):850-859. doi:10.1158/2159-8290
11. 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;25(13):3753-3758. doi:10.1158/1078-0432.CCR-18-4070
12. Clay R, Kipp BR, Jenkins S, et al. Computer-aided nodule assessment and risk yield (CANARY) may facilitate non-invasive prediction of EGFR mutation status in lung adenocarcinomas. Sci Rep. 2017;7(1):17620. doi:10.1038/s41598-017-17659-6

Performance

Method Description

Next-generation sequencing is performed to determine microsatellite instability status and evaluate the presence of a mutation in all coding regions of the *ALK, BRAF, EGFR, ERBB2, HRAS, KRAS, MDM2, MET* (including exon 14 skipping), *NRAS, RET, ROS1*, and *STK11* genes. See Targeted Genes and Methodology Details for MayoComplete Lung Cancer Mutations for details regarding the targeted gene regions evaluated 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
81457

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MCLNM	MayoComplete Lung Cancer Mutations	102042-9

Result ID	Test Result Name	Result LOINC® Value
617841	Result	82939-0
617842	Interpretation	69047-9
617843	Additional Information	48767-8
617844	Specimen	31208-2
617845	Tissue ID	80398-1
617846	Method	85069-3
617847	Disclaimer	62364-5
617848	Released By	18771-6