MAYO CLINIC LABORATORIES

Test Definition: LUCSF

LiquidHALLMARK CSF

Overview

Useful For

Genomic profiling of suspected primary brain tumors or brain or leptomeningeal metastases for predicting prognosis and identifying matched targeted therapies or emerging resistance mechanisms

This test is **not useful for** prenatal screening.

Genetics Test Information

This test uses amplicon-based next-generation sequencing (NGS) to determine single nucleotide variants (SNVs, including cis-trans), deletions and insertions (delins), copy number variations (CNVs), and microsatellite instability (MSI). Circulating tumor DNA (ctDNA) is used to detect somatic (ie, tumor specific) mutations in 81 genes.

Note: This test is performed to evaluate for somatic (ie, tumor-specific) mutations. Although germline (ie, inherited) alterations may be detected, this test cannot distinguish between germline variants and somatic mutations with absolute certainty.

Highlights

LiquidHALLMARK CSF is a sensitive next-generation sequencing assay that targets circulating tumor DNA in cerebrospinal fluid (CSF) in patients with suspected brain or leptomeningeal metastases. It can detect driver mutations and emerging mutations of therapeutic resistance to inform physicians of the appropriate targeted treatment selection.

Method Name

Amplicon-Based Next-Generation Sequencing

NY State Available

No

Specimen

Specimen Type

CSF

Shipping Instructions

Freeze the specimen immediately following collection. It is critical that the specimen remains frozen throughout the shipping process and is never thawed.

The collected specimen is stable for up to 30 days if stored frozen at the collection site.

Necessary Information

1. Order questions are required for testing to proceed.
If not ordering electronically, submit LiquidHALLMARK Patient Information with the specimen.
2. A pathology report is recommended. Testing may proceed without this information; however, it aids in providing a more thorough and accurate interpretation of results. Ordering healthcare professionals are strongly encouraged to provide the information and send it with the specimen.

Specimen Required

Supplies: Sterile Specimen Tube, 6 mL (T485)
Container/Tube: Sterile tube
Specimen Volume: 5 mL
Collection Instructions:
1. Perform lumbar puncture and discard the first 1 mL to 2 mL of cerebrospinal fluid (CSF).
2. Collect CSF directly into a sterile tube.
3. Inspect specimen for visible discoloration. **Specimen must be clear and colorless to perform testing.**
4. Freeze sample upright prior to placing in transport container.
5. Send frozen.

Forms

If not ordering electronically, complete, print, and send a LiquidHALLMARK Patient Information with the specimen.

Specimen Minimum Volume

1 mL

Reject Due To

Hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
CSF	Frozen	30 days	

Clinical & Interpretive

Clinical Information

LiquidHALLMARK CSF enables genomic profiling of cerebrospinal fluid (CSF) cell-free DNA to detect clinically relevant and actionable alterations associated with FDA-approved and emerging therapies, including key biomarkers such as *EGFR, BRAF, KRAS, ERBB2,* and *H3 (histone) gene mutations*, as well as other guideline-recommended targets.

In patients with suspected or confirmed CNS or leptomeningeal metastases, CSF-based analysis can complement tissue and plasma testing by improving detection of tumor-derived alterations within the central nervous system. Results may support therapy selection and provide insight into molecular evolution and treatment response over time.

This test is intended as an adjunct to standard diagnostic approaches and is not a substitute for primary diagnosis.

Reference Values

An interpretive report will be provided

Interpretation

The interpretation of molecular biomarker analysis includes an overview of the results and the associated prognostic and therapeutic implications.

Cautions

This report reflects the analysis of DNA from an extracted nucleic acid sample, and in very rare cases (for example, bone marrow transplant or recent blood transfusion) the analyzed DNA may not reflect the patient's genome, leading possibly to false negative and/or false positive results. Nucleic acid studies do not constitute a definitive test for the selected conditions in all individuals.

This circulating tumor (ct) DNA test is clinically validated for CSF specimens only.

It should be realized that there are possible sources of error. Errors can result from trace contamination, rare technical errors, rare genetic variants that interfere with analysis, recent scientific developments, and alternative classification systems.

Test sensitivity may be altered based on factors such as excessive cell lysis before processing, sampling during treatment, tissue heterogeneity, and the relative yield of circulating nucleic acids from sample.

Sensitivity of this test has been determined for the test methodology for a set of variants that do not necessarily include those identified in the report. Sensitivity and specificity data for all variants reported are not available. Where reported allele frequencies fall below 0.1% (single nucleotide variations/deletions-insertions), absolute number of variant reads supporting the call are considered, but specificity data is not available on this. Deletions or insertions involving more than 30 base pairs may not be reliably detected by the sequencing methodology. Although most of the intended targeted regions are sequenced in their entirety, some regions may be incompletely covered due to technical limitations. Therefore, absence of a detected variant in these regions and in regions not covered by this test does not exclude the presence of a disease-causing variant. Intronic variants and synonymous substitutions are not reported unless previously documented as clinically significant. Variants classified as benign or likely benign in ClinVar and/or variants with population allele frequency (in external or internal databases) of greater than 1% (non-founder mutations) are not reported.

This test is not intended for and cannot confirm germline status in any manner. Variants detected may be of tumor-derived somatic, germline, or non-tumor somatic origins, including mosaicism, clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited to, *ASXL1, ATM, CBL, DNMT3A, JAK2, MPL, MYD88, SF3B1, TET2, TP53,* and *U2AF1*. Clinical correlation is recommended. Genetic counseling may be considered if deemed appropriate clinically.

The absence of ctDNA findings may correlate with low systemic disease volume or disease that is being effectively treated. It is also possible that there are genomic alterations in targets not included in the panel or others not detectable

by this analysis due to inherent analytical limitations.

This test should be one of many aspects used by the treating healthcare professional to help with a diagnosis and treatment plan, but it is not a diagnosis itself. Clinical diagnosis provided by the treating healthcare professional is used to determine the relevant indication for determining appropriate clinical actionability/evidence and matching clinical trials, presentation of which may be adversely affected in cases of incomplete or incorrect diagnosis information provided. Any mention of pharmacologic agents or their on-label or off-label use should not be considered as a recommendation or endorsement for therapeutic use. Approved indications for the listed therapies may have additional criteria of medical and treatment history and combination chemotherapy. Percentage map is for visualization purposes only and is not drawn to scale. Clinical correlation is advised. Past treatment or mutation history is not being considered for selection of clinical trials presented. Clinical correlation and suitability with specific trial's inclusion and exclusion criteria are advised. Drug and clinical trial information are obtained from curated databases including NCI thesaurus and ClinicalTrials.gov. Clinical trial curated database is updated with trials verified within the last month. Tiering of clinical actionability/evidence associated with a drug recommendation may be updated in source data but not reflected as at the time of the report. For latest information, refer to the US Food and Drug Administration website and the respective source data websites for professional guidelines.
Lucence does not warrant that the data from such third-party databases, websites, or guidelines are accurate, complete, or up to date and excludes all liability for any loss or damage howsoever arising as a result of any reliance on the accuracy of the data.

Supportive Data

Test performance specifications are determined using commercial circulating tumor (ct) DNA standards, contrived samples including cell line DNA, and CSF clinical samples for specific variants at varying allele frequencies. Specificity has been determined for the entirety of bases targeted in the assay and is not variant- or hotspot-specific. The limit of detection for single nucleotide variations (SNVs)/deletions-insertions (delins) is determined to be 0.1% and 0.75% based on 25ng and 5ng input, respectively. For copy number alterations, the limit of detection has been determined to be 2-fold copy number gain/loss. The limit of detection for microsatellite instability is determined to be 5% with deletions-insertions in the microsatellite loci in the background of normal DNA.

Input DNA (ng)	Mutation class	Sensitivity by Mutant Allele Frequency					Specificity
		0.1%	0.5%	0.75%	2.5%	5%	
25	SNVs	>90%	>99%			>99%	>95%
	Indels	>93%	>99%			>99%	>91%
5	SNVs			>99%	>99%		>95%
	Indels			>98%	>99%		>91%

Clinical Reference

1. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2022;33(8):750-768. doi:10.1016/j.annonc.2022.05.520
2. Iams WT, Mackay M, Ben-Shachar R, et al. Concurrent tissue and circulating tumor DNA molecular profiling to detect guideline-based targeted mutations in a multicancer cohort. JAMA Netw Open. 2024;7(1):e2351700. Published 2024 Jan 2. doi:10.1001/jamanetworkopen.2023.51700
3. Poh J, Ngeow KC, Pek M, et al. Analytical and clinical validation of an amplicon-based next generation sequencing

assay for ultrasensitive detection of circulating tumor DNA. PLoS One. 2022;17(4):e0267389. Published 2022 Apr 29. doi:10.1371/journal.pone.0267389
4. Seoane J, De Mattos-Arruda L, Le Rhun E, Bardelli A, Weller M. Cerebrospinal fluid cell-free tumour DNA as a liquid biopsy for primary brain tumours and central nervous system metastases. Ann Oncol. 2019;30(2):211-218. doi:10.1093/annonc/mdy544

Performance

Method Description

Nucleic acid (cfDNA) is extracted from the cerebrospinal fluid (CSF) sample. The extracted DNA undergoes sequencing library construction for genes targeted in the LiquidHALLMARK CSF assay. Quality and concentration of constructed libraries are determined and then sequenced on an Illumina NextSeq/NovaSeq instrument with 2x150 pair-end reads. Targeted regions listed in the LiquidHALLMARK Targets by Cancer Type (ctDNA) or a relevant subset of the list, selected to maximize detections of known hotspot mutations, are analyzed for sequence variants. Six microsatellite loci (BAT25, BAT26, NR21, NR24, NR27, MONO27) are analyzed for deletions-insertions in homopolymeric regions. Samples with microsatellite instability (MSI) detected in two or more of six sites are considered MSI-High (MSI-H) and those with MSI detected in one of six sites are considered MSI-Low (MSI-L). Sequences are aligned to reference sequences based on human genome build GRCh37/UCSC hg19. Data is analyzed using in-house bioinformatics pipelines, and proprietary sequencing error-correction methodology is applied on raw sequencing data. Copy number changes are calculated based on adjusted read count, and its variation from normalized baseline read count determined across control samples. All sequence alterations are described according to the Human Genome Variation Society (HGVS) nomenclature guidelines as published.(1) Clinical actionability of genomic findings is determined based on curated databases from publicly available data sources, including peer-reviewed publications of genomic alterations and biomarkers and associated drugs. Tiering of clinical actionability is based on Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists consensus recommendation (2,3) where clinical actionability are based on Tier 1 evidence level (US Food and Drug Administration [FDA], guidelines, Phase III trials, well-powered studies with expert consensus). Drug and clinical trial information are obtained from curated databases including NCI thesaurus and ClinicalTrials.gov.

1. den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. Hum Mutat. 2016;37(6):564-569. doi:10.1002/humu.22981
2. 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 Diagn. 2017;19(1):4-23. doi:10.1016/j.jmoldx.2016.10.002
3. Wagner AH, Walsh B, Mayfield G, et al. A harmonized meta-knowledgebase of clinical interpretations of somatic genomic variants in cancer. Nat Genet. 2020;52(4):448-457. doi:10.1038/s41588-020-0603-8

PDF Report

Referral

Day(s) Performed

Monday through Friday

Report Available
8 to 12 days

Performing Laboratory Location
Lucence Health, Inc.

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 clinical test was developed and its performance characteristics determined by Lucence Health Inc. It has not been cleared or approved by the US Food and Drug Administration (FDA). The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes and should not be regarded as investigational or for research, unless otherwise stated in the report. Lucence Health Inc. is a Clinical Laboratory Improvement Amendments (CLIA)-certified clinical diagnostic laboratory (CLIA ID Number: 05D2200843) and is accredited to College of American Pathologists (CAP) laboratory quality standards.

CPT Code Information
81455

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LUCSF	LiquidHALLMARK CSF	Not Provided

Result ID	Test Result Name	Result LOINC® Value
LU003	LiquidHALLMARK CSF	Not Provided