Test Definition: LUCHM

LiquidHALLMARK ctDNA and ctRNA

Overview

Useful For

As an alternative to invasive tissue biopsies to assist in tumor profiling for diagnosis, predicting prognosis, and identifying targeted therapies for the treatment and management of patients with a solid tumor

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), microsatellite instability (MSI) and fusions. Circulating tumor DNA (ctDNA) is used to detect sequence variants in 80 genes, fusions in 10 genes, and somatic mutations. Circulating tumor RNA (ctRNA) is used to analyze 10 ctRNA targets for actionable and emerging fusions. See LiquidHALLMARK Targets by Cancer Type for details regarding genes interrogated.

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

Highlights

LiquidHALLMARK is a sensitive next-generation sequencing assay targeting both circulating tumor DNA and RNA to profile a patient's unique cancer. With a blood draw, LiquidHALLMARK provides important information for cancer care especially when tissue by invasive biopsy is insufficient or inaccessible.

LiquidHALLMARK targets genes that are commonly associated with 15 cancers, including lung, breast and colon cancer.

Method Name

Amplicon-Based Next-Generation Sequencing

NY State Available

No

Specimen

Specimen Type

WB Streck

Shipping Instructions

Specimen must be received at Mayo Clinic Laboratories within 4 days of collection.

Necessary Information

1. Answers to the Order Questions are required for testing to proceed.

If needed, use the LiquidHALLMARK Patient Information form to provide information to the ordering laboratory. The

answers on this form should be added to MayoAccess or MayoLink when ordering the test.
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 with the specimen.

Specimen Required

Specimen Type: Whole blood
Supplies: Streck Tan Top Tube Kit (T715)
Container/Tube: Two 10-mL Streck cell-free DNA (cfDNA) blood collection tubes
Specimen Volume: 20 mL; 10 mL in two Streck tubes
Collection Instructions:
1. Invert several times to mix blood.
2. Send whole blood specimen in original tube. **Do not centrifuge or aliquot.**

Forms

LiquidHALLMARK Patient Information

Specimen Minimum Volume

See Specimen Required

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject
Gross icterus	Reject
One specimen tube received	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
WB Streck	Ambient	4 days	Streck Black/Tan top

Clinical & Interpretive

Clinical Information

LiquidHALLMARK detects clinically significant and actionable alterations associated with US Food and Drug Administration (FDA)-approved and emerging therapies, including tissue-agnostic targets *BRAF*, *RET*, *NTRK*, and *MSI*, and guideline-recommended biomarkers. The identification of these alterations allows healthcare professionals to make informed decisions to guide care from diagnosis and initial therapy selection, monitoring of therapy response, to the detection of emergent mutations that drive disease burden over time. The use of next-generation sequencing of circulating tumor DNA and RNA enables both minimal invasive testing and broad genomic coverage to maximize therapeutic benefit to patients.

This test is intended for the genomic profiling of solid tumors via the use of circulating-free DNA and RNA.

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 report reflects the analysis of DNA and RNA 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 to possible false-negative 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 and ctRNA test is clinically validated for plasma specimens only. Other specimen types, including but not limited to pleural effusions, pericardial effusions, and cerebrospinal fluid, have not been validated.

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. Lipemic plasma specimens may also result in reduced assay sensitivity or assay failure.

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) or 0.5% (DNA fusion), 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.

For cases where no genomic alterations and no ctRNA findings are identified, the absence of plasma ctDNA alterations

and ctRNA findings may correlate with low systemic disease volume or disease that is being effectively treated. It is also possible that there are genomic/ctRNA alterations in targets not included in the panel or others not detectable by this analysis due to inherent analytical limitations. Further clinical correlation is advised, with consideration of follow-up tissue or plasma testing.

Results for fusions, splice and exon-skipping variants from ctDNA and ctRNA assay components may not be fully concordant due to differing test sensitivities and differing limits of detection for ctDNA and ctRNA assays, differing target gene coverage in each assay, and sample-specific variations in levels of fusion, splice and exon skipping variant RNA transcripts depending on transcription rates of DNA to RNA.

For the ctRNA component, this test has been validated for fusions, splice and exon-skipping variants in *ALK*, *FGFR2*, *FGFR3*, *MET*, *NTRK1*, *NTRK2*, *RET*, *ROS1*, and *TMPRSS2*. The clinical significance of other findings presented in this section has not been established. These other findings are evaluated and reported as part of the standard workflow.

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 plasma 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) and fusion is determined to be 0.1% and 0.5%, respectively. For copy number alterations, the limit of detection has been determined to be an excess of 0.6 copy for a copy number gain of 1.3-fold (2 vs. 2.6), and a deficiency of 0.71 copy for a copy number loss of 1.4-fold (2 vs. 1.43). The limit of detection for microsatellite instability is determined to be 5% DNA with deletions-insertions in the microsatellite loci in the background of normal DNA.

Mutant allele frequency	**0.1%**		**0.5%**		**1%**		**5%**	
	Sensitivit	Specificit	Sensitivit	Specificit	Sensitivit	Specificit	Sensitivit	Specificity

		y	y	y	y	y	y	y	
Mutation class	**SNVs**	>90%	>94%	>99%	>99%	-	-	>99%	>99%
	Delins	>92%	>90%	>99%	>99%	-	-	>99%	>99%
	Fusions	-	-	>91%	>99%	>93%	>99%	>99%	>99%

For ctRNA fusions, test performance specifications were determined using commercial RNA fusion standards, contrived samples including cell line RNA, and plasma clinical samples with orthogonal characterization of fusions from ctDNA. The limit of detection for ctRNA fusions was determined to be 10 copies.

RNA fusion copy number	10		100	
	Sensitivity	Specificity	Sensitivity	Specificity
	>99%	>99%	>99%	>99%

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. Benayed R, Offin M, Mullaney K, et al. High yield of RNA sequencing for targetable kinase fusions in lung adenocarcinomas with no mitogenic driver alteration detected by DNA sequencing and low tumor mutation burden. Clin Cancer Res. 2019;25(15):4712-4722. doi:10.1158/1078-0432.CCR-19-0225

4. Heeke S, Gandhi S, Tran HT, et al. Brief Report: Longitudinal tracking of ALK rearranged non-small cell lung cancer from plasma using circulating-tumor RNA and circulating-tumor DNA. JTO Clin Res Rep. 2025;in press. doi:10.1016/j.jtocrr.2025.100795

5. 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

Performance

Method Description

Plasma nucleic acid (cell-free [cf] DNA and cfRNA) is extracted from blood. The extracted DNA undergoes sequencing library construction for genes targeted in the LiquidHALLMARK assay. Quality and concentration of constructed libraries are determined and then sequenced on an Illumina NextSeq 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. Targeted regions are analyzed for sequence variants and/or structural rearrangement (fusions). 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.

For ctRNA testing, the extracted RNA undergoes reverse transcription and sequencing library construction for genes targets in the ctRNA test component of the LiquidHALLMARK assay. Quality and concentration of constructed libraries are determined and then sequenced on an Illumina NextSeq instrument with 2x150 paired-end reads. Primers for selected exons of the tested genes and selected exons of their fusion partner genes, landing upstream or downstream of the exon junctions, are used to identify presence of fusions, splice and exon skipping variants among the target genes. Sequences are aligned to reference sequences based on human genome build GRCh37/UCSC hg19, and fusions, splice and exon skipping variants detected based on split read sequences spanning non-contiguous genome segments corresponding to exon boundaries of genes targeted. Data is analyzed using in-house bioinformatics pipelines. All ctRNA fusion, splice and exon skipping variants are described with the exonic breakpoints of the genes involved. All sequence alterations are described according to GeneA-GeneB nomenclature. The same clinical evidence and clinical recommendations apply to fusion findings from ctRNA and genomic findings from ctDNA.

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

0571U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LUCHM	LiquidHALLMARK ctDNA and ctRNA	Not Provided

Result ID	Test Result Name	Result LOINC® Value
LU001	LiquidHALLMARK ctDNA and ctRNA	Not Provided
LUC01	Original Diagnosis Date	No LOINC Needed
LUC02	Diagnosis	No LOINC Needed
LUC03	Subtype If Applicable	No LOINC Needed
LUC04	Disease Stage	No LOINC Needed
LUC05	Current Therapy and Response	No LOINC Needed
LUC06	Disease Status	No LOINC Needed
LUC07	ICD-10 Codes	No LOINC Needed