MAYO CLINIC LABORATORIES

Test Definition: MSCSF

Bacteria, Virus, Fungus, and Parasite Metagenomic Sequencing, Spinal Fluid

Overview

Useful For

Detecting and identifying pathogenic organisms including bacteria, fungi, DNA viruses, RNA viruses, and parasites in cerebrospinal fluid

This test is **not recommended** as a test of cure because nucleic acids may persist after successful treatment.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
SPID2	Specimen Identification by PCR	No, (Bill Only)	No

Testing Algorithm

For more information see Meningitis/Encephalitis Panel Algorithm.

Special Instructions

- Meningitis/Encephalitis Panel Algorithm
- Bacteria, Virus, Fungus, and Parasite Metagenomic Sequencing Patient Information

Highlights

This test detects and identifies bacteria, DNA and RNA viruses, fungi, and parasites in cerebrospinal fluid using next-generation sequencing.

Method Name

Metagenomic Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

CSF

Necessary Information

The following ask-at-order entry question must be answered at the time of test ordering:

Which CSF Collection Vial Number Sent (Required, Vial 1 is not acceptable)

Note: Answers may include "Vial 2", " Vial 3", or " Vial 4".
Test requests for specimens from "Vial 1" will be canceled. Test requests for submitted specimens with no response or response that does not specify the collection vial number will result in testing delays while the submitting facility is contacted to provide the information.

Specimen Required

Container/Tube: Sterile vial
Specimen Volume: 2 mL
Collection Instructions:
1. Masks should be worn by those collecting and processing specimens for this assay.
2. Handle all vials under sterile technique when open to the air.
3. A separate collection vial of CSF is preferred.
4. Submit specimen from collection vial 2, 3, or 4, as specimens from vial 1 are not acceptable.
5. Indicate on the label which vial is being submitted.
6. **Do not centrifuge or heat inactivate**.
7. **Shunt fluid and ventricular fluid specimens are not acceptable**.

Forms

1. If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen:
2. Bacteria, Virus, Fungus, and Parasite Metagenomic Sequencing Patient Information

Specimen Minimum Volume

0.5 mL

Reject Due To

Gross hemolysis	Reject
Shunt fluid	Reject
Ventricular fluid	Reject
Heat-inactivated specimen	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
CSF	Frozen (preferred)	21 days	
	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

The target population is patients with suspected, but undiagnosed, central nervous system infection. Infection of the central nervous system is a potentially life-threating condition that requires rapid diagnosis and clinical treatment. Infections of the central nervous system have broad pathogen etiology, including bacteria, fungi, viruses, and parasites. The breadth of causative agents challenges diagnostic test ordering and pathogen identification. Current clinical diagnostic methods, such as culture and specific-polymerase chain reaction assays, have limitations in the ability to detect non-viable organisms, or nucleic acids that are not targeted by specific assays, respectively. An unbiased metagenomic sequencing approach overcomes diagnostic test limitations by interrogating microbiota without bias towards any specific microorganisms. Bioinformatic analysis of the resultant large sequencing dataset enables identification of a diversity of pathogens in this assay. The test can identify multiple pathogens in a single specimen if present.

Reference Values

Negative.
No pathogenic DNA virus detected.
No pathogenic RNA virus detected.
No pathogenic parasite detected.
No pathogenic bacterium detected.
No pathogenic fungus detected.

Interpretation

A positive result indicates that nucleic acid of one or more potentially pathogenic microorganisms was detected. A negative result indicates absence of detectable nucleic acids from potentially pathogenic bacteria, fungi, viruses, or parasites. A negative result does not rule the presence of a pathogen due lack of a reference sequence in the database used, the presence of microbial nucleic acids in quantities lower than the limit of detection of the assay, or inhibition from high levels of competing human nucleic acid. If testing indicates inhibition, testing will be repeated. If inhibition is again detected, the result will be reported with a comment indicating that inhibition was present.

Cautions

This test does not detect prions. False-positive results are possible if specimens are contaminated with microbial nucleic acids from environmental contamination, patient microbiota (eg, from the skin) or microbiota of those collecting or processing the specimen.

High levels of human nucleic acids in specimens can decrease test sensitivity for microorganism detection and result in sequencing competition interpreted as inhibition.

Not all infecting central nervous system pathogens are detectable in cerebrospinal fluid (CSF) by this test.

Results are intended to be used in conjunction with clinical findings. This test is only validated for CSF collected via lumbar puncture.

One organism per class of pathogen was used to establish the limits of detection, providing an estimation of the assay's capability to detect each class of pathogen. Actual detection capability may vary by specific organism based on genome size and other factors.

Epstein Barr virus detection:

Clinical significance of Epstein Barr virus detection in CSF is uncertain and may suggest latent infection of white blood cells, inflammatory reactivation, post-transplant lymphoproliferative disorder, or neurologic disease.

Cytomegalovirus detection:
Clinical significance of cytomegalovirus detection in CSF is uncertain and may suggest latent infection of white blood cells, inflammatory reactivation, or neurologic disease.

Human Herpes virus 6 detection:
Clinical significance of human herpes virus 6 (HHV-6) detection in CSF is uncertain and may suggest latent infection of white blood cells, inflammatory reactivation, chromosomally integrated HHV-6, or neurologic disease.

HIV-1 detection:
HIV-1 can be detected in CSF of HIV-positive individuals; clinical significance is uncertain.

Supportive Data

Fifty-six cerebrospinal fluid (CSF) specimens positive for a microorganism using a specific polymerase chain reaction (PCR), culture, serology, and/or multiplex PCR were tested. In addition, 30 CSF samples negative by multiplex PCR testing were evaluated. Overall sensitivity of the assay was 86%, and specificity 100% compared to predicate testing.

Clinical Reference

Rodino KG, Toledano M, Norgan AP, et al. Retrospective review of clinical utility of shotgun metagenomic sequencing testing of cerebrospinal fluid from a U.S. tertiary care medical center. J Clin Microbiol. 2020;58(12):e01729-20. doi:10.1128/JCM.01729-20

Performance

Method Description

Cerebrospinal fluid is collected in a sterile container and the test portion aliquoted and bead beat. Specimens are separated into equal volume RNA and DNA pools following total nucleic acid isolation and spiked with internal controls. The RNA pool is treated before undergoing reverse transcription to convert total RNA into complementary DNA. RNA and DNA are then prepared for sequencing through size-selection, adapter addition, and addition of unique dual indices. Sequencing is performed on an Illumina NextSeq 1000. Run controls consist of two negative extraction controls, a difficult to lyse DNA positive extraction control, an RNA positive extraction control, and internal DNA and RNA phage inhibition controls for each sample.(Unpublished Mayo method)

Bioinformatic analysis uses a cloud-based solution for data analysis. This software provides pathogen identification from complex specimen types. The pipeline includes sequencing QC, human sequence deletion, and organism sequence alignment. The website includes a graphical user interface, cloud-based data upload and analyses, and alignment of generated sequencing data to National Center for Biotechnology Information’s pathogen reference database.

PDF Report

No

Day(s) Performed
Monday through Friday

Report Available
7 to 14 days

Specimen Retention Time
14 days

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
0480U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MSCSF	Metagenomic Sequencing, CSF	103566-6

Result ID	Test Result Name	Result LOINC® Value
MSCSF	Metagenomic Sequencing, CSF	103566-6