MAYO CLINIC LABORATORIES

Test Definition: BRBPS

Broad Range Bacterial PCR and Sequencing, Varies

Overview

Useful For

Detecting and identifying bacteria (including mycobacteria) from normally sterile sources, including synovial fluid; body fluids such as pleural, peritoneal, and pericardial fluids, cerebrospinal fluid; and both fresh and formalin-fixed paraffin-embedded tissues

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

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
ISBA	Bacterial Ident by Sequencing	No, (Bill Only)	No
ISNGS	Ident by Next Generation Sequencing	No, (Bill Only)	No
SPID2	Specimen Identification by PCR	No, (Bill Only)	No
CSFME	Meningitis Encephalitis Panel, PCR	Yes	No
JIP	Joint Infect Panel PCR, Synovial Fl	Yes	No

Testing Algorithm

If polymerase chain reaction (PCR) testing is negative, no sequencing is performed, and the test resulted as negative.

If PCR testing is positive, sequencing is performed. Strong positive results are first submitted to Sanger sequencing, which can yield results in as few as 4 days. Weak positive results, or Sanger sequencing results that are mixed, are submitted to next-generation sequencing.

The following algorithms are available:
-Infective Endocarditis: Diagnostic Testing for Identification of Microbiological Etiology
-Meningitis/Encephalitis Panel Algorithm

Special Instructions

- Infective Endocarditis: Diagnostic Testing for Identification of Microbiological Etiology
- Meningitis/Encephalitis Panel Algorithm

Highlights

This test is used for detection and identification of bacteria (including mycobacteria) in normally sterile specimens.

This test is optimal for situations in which bacteria (including mycobacteria) are visualized in the specimen, but other laboratory methods have failed to yield a diagnosis.

Method Name
Polymerase Chain Reaction (PCR) followed by Sequencing

NY State Available
Yes

Specimen

Specimen Type
Varies

Necessary Information
Specimen source is required.

Specimen Required
Fresh tissue is preferred over formalin-fixed, paraffin-embedded tissue.

Submit only 1 of the following specimens:

Preferred Specimen Type:

Specimen Type: Fresh tissue or biopsy
Sources: Normally sterile tissue such as bone, lymph node, joint, heart valve, brain, viscera, organ, lung, prostate
Container/Tube: Sterile container
Specimen Volume: Entire collection or 5 mm(3)-approximately the size of a pencil eraser
Collection Instructions:
1. Collect fresh tissue specimen.
2. Submit tissue only, do not add fluid to tissue.
3. Freeze specimen.
Specimen Stability Information: Frozen (preferred) <21 days/Refrigerated <21 days

Alternate Specimen Types:

Preferred: Paraffin-embedded tissue block
Supplies: Tissue Block Container (T553)
Specimen Type: Formalin-fixed, paraffin-embedded (FFPE) tissue block
Sources: Normally sterile or deep tissues such as bone, lymph node, joint, heart valve, brain, viscera, organ, lung, prostate

Container/Tube: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block to be cut and returned.
Specimen Stability Information: Ambient (preferred)/Refrigerated

Acceptable: Paraffin-embedded tissue block
Specimen Type: Section (scrolls) of FFPE tissue block
Sources: Normally sterile or deep tissues such as bone, lymph node, joint, heart valve, brain, viscera, organ, lung, prostate
Container/Tube: Sterile container for each individual cut section (scroll)
Collection Instructions: Perform microtomy and prepare five separate 10-micron sections. **Each section (scroll) must be placed in a separate sterile container for submission.**
Specimen Stability Information: Ambient (preferred)/Refrigerated

Specimen Type: Fluid
Sources: Normally sterile body fluids such as vitreous humor, pleural, abdominal, peritoneal, ascites, pericardial, pelvic, prostatic
Container/Tube: Screw-capped, sterile container
Specimen Volume: 1 mL
Collection Instructions:
1. Collect fresh fluid specimen.
2. Freeze specimen.
Specimen Stability Information: Frozen (preferred) <21 days/Refrigerated <21 days

Specimen Type: Spinal fluid
Container/Tube: Screw-capped, sterile container
Specimen Volume: 1 mL
Collection Instructions:
1. Collect fresh spinal fluid (CSF) specimen using sterile technique.
2. Submit specimen from collection vial 2 or higher, specimens in vial 1 are not acceptable.
3. Indicate on the label which vial is being submitted.
4. CSF collected via shunt and ventricular fluid are also acceptable. Label tube with applicable collection information if submitting one of these specimens.
Specimen Stability Information: Frozen (preferred) <21 days/Refrigerated <21 days

Specimen Type: Synovial fluid
Container/Tube:
Preferred: Red top or sterile container
Acceptable: Lavender top (EDTA), pink top (EDTA), royal blue top (EDTA), or sterile vial containing EDTA-derived aliquot
Specimen Volume: 1 mL
Collection Instructions: Send specimen in original tube (preferred).
Specimen Stability Information: Frozen (preferred) <21 days/Refrigerated <21 days

Forms

If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

Fluid: 0.5 mL; Fresh tissue or biopsy: 5 mm(3); Paraffin-embedded tissue block: two 10-micron sections

Reject Due To

Specimen received in anaerobe vial Tissue received in any fluid (saline, broth, formalin, formaldehyde, acetone, etc) Wrapping (gauze, drapes, etc) Blood Culture bottles (Bactec FX and/or BacT/ALERT bottles) Bone marrow Decalcified bone Slides Skin biopsy Colon biopsy Formalin-fixed paraffin-embedded body fluid	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical & Interpretive

Clinical Information

Cultures from patients with suspected bacterial infection involving normally sterile sites may fail to provide bacterial

(including mycobacterial) growth for identification due to the presence of fastidious or slow-growing bacteria or because of antecedent antimicrobial chemotherapy. Polymerase chain reaction amplification of a portion of the 16S ribosomal RNA (rRNA) gene followed by sequencing of the amplified product can be used to detect bacterial (including mycobacterial) nucleic acids in such situations, enabling a diagnosis. Sterile sources accepted for testing may have more than one bacterial species present or the presence of copy variants of the 16S rRNA gene within a single bacterial species, confounding Sanger sequencing analysis. Next-generation sequencing can be useful in such cases. Ideal specimens are those in which bacteria (includes mycobacteria) are visualized by microscopy. Heart valves from patients with endocarditis with positive Gram stains are, for example, especially suitable.

Reference Values

No bacterial DNA detected

Interpretation

A positive broad-range polymerase chain reaction (PCR)/sequencing result indicates that bacterial nucleic acid of the specified organism was detected, which may be due to bacterial infection or environmental or contaminating nucleic acids in the specimen.

A negative broad-range PCR/sequencing result indicates the absence of detectable bacterial (including mycobacterial) nucleic acids in the specimen but does not rule out false-negative results that may occur due to sampling error, sequence variability underlying the primers, the presence of bacterial nucleic acids in quantities below the limit of detection of the assay, or inhibition of PCR. If PCR testing appears to be negative but there is evidence of PCR inhibition, testing will be repeated. If inhibition is again detected, the result will be reported as "PCR inhibition present."

Cautions

This test does not detect nonbacterial organisms (eg, viruses, fungi, helminths, protozoa), but does detect mycobacteria.

False-positive results are theoretically possible if patient specimens or collection containers are contaminated with bacterial nucleic acids either from the environment or from patient microbiota (eg, skin microbiota contamination).

This test is validated for normally sterile sources.

In extenuating circumstances, sequencing, especially next-generation sequencing, may be associated with an extended turnaround time, approaching, or possibly exceeding, the published maximum report available time (28 days). This typically happens if repeat testing is needed, information about the specimen is being sought, or orthogonal testing is being performed.

Supportive Data

One hundred thirty positive patient specimens were available for accuracy studies and correlated with results of culture, organism-specific polymerase chain reaction (PCR), or previous broad-range bacterial PCR and sequencing. In addition, 63 negative samples from previous Sanger sequence-based testing were used in verification. All samples were tested with both Sanger and next-generation sequencing (NGS) technologies enabling resolution of poor-quality Sanger results and identifying polybacterial presence in some samples. Using criteria established in verification, analytical sensitivity of the assay is 99% and specificity is 97%. Some samples were spiked with gram-negative or gram-positive bacteria due to the scarcity of clinically positive samples. Testing demonstrated 100% correlation with expected results from spiked material.

The limit of detection was less than 65 colony forming units per PCR reaction for all sources as determined by spiking *Streptococcus gallolyticus* and *Escherichia coli* into PCR-negative fresh tissue, synovial fluid, formalin-fixed, paraffin-embedded tissue, sonicate fluid, body fluid, and cerebrospinal fluid.

Specificity was tested using a panel of 10 nucleic acid extracts from viral, fungal, and parasitic organisms. No cross-reactivity to these organisms was observed.

Inclusivity studies were performed by amplifying 42 genomic DNA samples representing diverse types of bacteria (including mycobacteria) expected to be present in the specimen types acceptable for this assay. All bacteria and mycobacteria were detected and correctly identified by both Sanger and NGS.

An additional study of 15 specimens previously characterized only as polybacterial revealed the ability of NGS to detect and differentiate multiple bacteria for reporting. The laboratory section director is responsible for reporting of polybacterial results.

Clinical Reference

1. Virk A, Pritt B, Patel R, et al. *Mycobacterium lepromatosis* lepromatous leprosy in US citizen who traveled to disease-endemic areas. Emerg Infect Dis. 2017;23(11):1864-1866. doi:10.3201/eid2311.171104
2. Liesman RM, Pritt BS, Maleszewski JJ, Patel R. Laboratory diagnosis of infective endocarditis. J Clin Microbiol. 2017;55(9):2599-2608. doi:10.1128/JCM.00635-17
3. Ramakrishna JM, Libertin CR, Yang JN, Diaz MA, Nengue AL, Patel R. 16S rRNA gene PCR/sequencing of cerebrospinal fluid in the diagnosis of post-operative meningitis. Access Microbiology. 2020;2(2):acmii.0.000100
4. Alvarez Otero J, Mandrekar J, Wolf MJ, et al. Pleural space infection microbiology as assessed using a clinically targeted sequencing-based assay: *Fusobacterium nucleatum* group, *Streptococcus intermedius*, and oral normal microbiota are the most common bacteria identified in community-acquired pleural space infections. J Clin Microbiol. 2024;62(12):00694-24-s0001
5. Azad MA, Wolf MJ, Strasburg AP, et al. Comparison of the BioFire Joint Infection Panel to 16S ribosomal RNA gene-based targeted metagenomic sequencing for testing synovial fluid from patients with knee arthroplasty failure. J Clin Microbiol. 2022;60(12):e0112622. doi:10.1128/jcm.01126-22
6. Fowler VG, Durack DT, Selton-Suty C, et al. The 2023 Duke-International Society for Cardiovascular Infectious Diseases criteria for infective endocarditis: Updating the modified Duke criteria [published correction appears in Clin Infect Dis. 2023 Oct 13;77(8):1222. doi: 10.1093/cid/ciad510]. Clin Infect Dis. 2023;77(4):518-526. doi:10.1093/cid/ciad271
7. Flurin L, Wolf MJ, Mutchler MM, Daniels ML, Wengenack NL, Patel R. Targeted metagenomic sequencing-based approach applied to 2146 tissue and body fluid samples in routine clinical practice. Clin Infect Dis. 2022;75(10):1800-1808. doi:10.1093/cid/ciac247
8. Hong HL, Flurin L, Greenwood-Quaintance KE, et al. 16S rRNA gene PCR/sequencing of heart valves for diagnosis of infective endocarditis in routine clinical practice. J Clin Microbiol. 2023;61(8):e0034123. doi:10.1128/jcm.00341-23

Performance

Method Description

This test utilizes specimen processing, DNA extraction, and polymerase chain reaction (PCR) of a highly variable fragment of the 16S ribosomal RNA (rRNA) gene. The variability of the targeted V1-V3 region allows for taxonomically specific reporting. If positive by PCR based on signal strength, the amplified DNA is sequenced to obtain identification of the source organism. If PCR is negative, no sequencing is performed. PCR inhibition is detected with a second PCR reaction and amplification is performed on a LightCycler. Only high-quality consensus sequence of 400 base pairs or more (usable data for both forward and reverse direction) is used for Sanger sequencing identification. If sequence data is not interpretable using Sanger sequencing, or the PCR signal is weak, but present, next-generation sequencing (NGS) is performed. Quality filtering is performed for NGS and only results with 100X coverage are used in analysis. Positive and negative controls are used throughout all processes to ensure assay performance. Sequence quality (specimen score) and data analysis for organism identification is accomplished with Pathogenomix RipSeq software.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
14 to 28 days

Specimen Retention Time
1 week

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
87801-Broad Range Bacterial PCR and Sequencing
87798-Bacterial Ident by Sequencing (if appropriate)
87798-Specimen Identification by PCR (if appropriate)
87798-Ident by Next Generation Sequencing (if appropriate)

87483-Meningitis Encephalitis Panel, PCR (if appropriate)
87999-Joint Infection Panel, PCR, Synovial Fluid (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BRBPS	Broad Range Bacteria PCR+Sequencing	76575-0

Result ID	Test Result Name	Result LOINC® Value
BRBPS	Broad Range Bacteria PCR+Sequencing	76575-0