

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 (CSF); and both fresh and formalin-fixed paraffin-embedded (FFPE) tissues

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

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.

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
ISBA	Bacterial Ident by Sequencing	No, (Bill Only)	No
PCRID	Identification by PCR	No, (Bill Only)	No

Testing Algorithm

If polymerase chain reaction (PCR) testing is positive, then reflex sequencing will be performed at an additional charge.

If PCR is negative, no sequencing will be performed.

The following algorithms are available in Special Instructions:

[-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](#)

Method Name

16S Ribosomal RNA Gene Polymerase Chain Reaction (PCR) followed by Sequencing

NY State Available

Yes

Specimen

Specimen Type

Varies

Specimen Required

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

Submit only 1 of the following specimens:

Preferred:

Specimen Type: Fresh tissue or biopsy

Sources: Normally sterile tissue such as bone, lymph node, joint, heart valve, brain, viscera, organ, lung

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 <14 days (preferred)/Refrigerated <14 days

Alternate:

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

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: FFPE tissue block

Sources: Normally sterile or deep tissues such as bone, lymph node, joint, heart valve, brain, viscera, organ, lung

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 cerebrospinal, vitreous humor, pleural, abdominal, peritoneal, ascites, pericardial, pelvic

Container/Tube: Screw-capped, sterile container

Specimen Volume: 1 mL

Collection Instructions:

1. Collect fresh fluid specimen.
2. Freeze specimen.

Specimen Stability Information: Frozen <14 days(preferred)/Refrigerated <14 days

Specimen Type: Synovial fluid

Container/Tube:

Preferred: Red clot tube (no anticoagulant) 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 <14 days (preferred)/ Refrigerated <14 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

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Tissue in formalin, formaldehyde, acetone, or any other fluid Bone marrow Decalcified bone Slides	Reject
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Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Varies		

Clinical and 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 as a result of antecedent antimicrobial chemotherapy. Polymerase chain reaction (PCR) amplification of a portion of the 16S ribosomal RNA 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 (NGS) 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(s) 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 less than 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 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.

Supportive Data

One hundred and 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, sensitivity of the assay is 99% and specificity is 97%. Some samples were spiked with Gram-negative or -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 (CFU)/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 (FFPE) tissue, sonicate fluid, body fluid, and cerebrospinal fluid.Â

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. Oyvind K, Simmon K, Karaca D, Langeland N, Wiker HG: Dual priming oligonucleotides for broad-range amplification of the bacterial 16S rRNA gene directly from human clinical specimens. J Clin Microbiol. 2012;50(4):1289-1294 doi: 10.1128/JCM.06269-11
2. Gomez E, Cazanave C, Cunningham SA, et al: Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. J Clin Microbiol. 2012;50:3501-3508
3. 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 Nov; 23(11):1864-1866 doi: 10.3201/eid2311.171104
4. Liesman RM, Pritt BS, Maleszewski JJ, Patel R: Laboratory diagnosis of infective endocarditis. J Clin Microbiol. 2017 Sep;55(9):2599-2608 doi: 10.1128/JCM.00635-17
5. 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 Feb 3;2 doi: 10.1099/acmi.0.000100

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 bp 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, 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) and Time(s) Test Performed

Monday through Friday

Analytic Time

7 days

Maximum Laboratory Time

15 days

Specimen Retention Time

1 week

Performing Laboratory Location

Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

87801-Broad Range Bacterial PCR and Sequencing

87798-Bacterial Ident by Sequencing (if appropriate)

87150-Identification by PCR (if appropriate)

81479Ident by Next Generation Sequencing (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