Test Definition: BRBST

Tickborne Bacterial, PCR and Sequencing, Blood

Overview

Useful For

Detecting and identifying pathogenic tickborne bacteria infecting normally sterile whole blood

Potential detection of bacteria that cause similar illnesses to tickborne infections

This test **should not be used** as first tier test. It should only be used when routine testing is negative.

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
SPID2	Specimen Identification by PCR	No, (Bill Only)	No

Testing Algorithm

For information see Acute Tick-borne Disease Algorithm.

Special Instructions

- Acute Tick-borne Disease Testing Algorithm for Mayo Clinic

Highlights

This test is used for detection and identification of pathogenic tickborne bacteria or organisms that have a similar clinical presentation (eg, Q-fever due to *Coxiella burnetii*, leptospirosis) infecting normally sterile whole blood.

This test is optimal for situations in which tickborne bacterial infection is suspected, but other laboratory methods have failed to yield a diagnosis.

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Necessary Information

Specimen source is required.

Specimen Required

Container/Tube:

Preferred: Lavender top (EDTA)

Acceptable: Royal blue top (EDTA), pink top (EDTA), or sterile vial containing EDTA-derived aliquot

Specimen Volume: 1 mL

Collection Instructions: If not submitting in original vial, mix well before transferring to a sterile vial.

Specimen Minimum Volume

0.5 mL

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated (preferred)	14 days	
	Frozen	14 days	

Clinical & Interpretive

Clinical Information

The target population is patients with suspected, but undiagnosed, tickborne bacterial infection involving normally sterile whole blood. Polymerase chain reaction (PCR) amplification of a portion of the 16S ribosomal RNA gene followed by next-generation sequencing of the amplified product can be used to detect tickborne bacterial nucleic acids in such situations, enabling a diagnosis. Ideal specimens are those that specific tickborne PCR tests or blood culture have not resulted in identifiable causative infectious agents. Due to the complexity of this test, the suspected tickborne disease testing algorithm will reflex to this assay only if specific-PCR tests are negative. The test is designed to identify mono-bacterial or poly-bacterial tickborne infections.

Reference Values

No tickborne bacterial DNA detected

Interpretation

A positive broad-range polymerase chain reaction (PCR)/sequencing result indicates that tickborne bacterial nucleic acid was detected.

A negative sequencing result indicates the absence of detectable bacterial 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 amplification. If testing shows evidence of PCR inhibition, it 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).

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

This test is validated for whole blood only.

Supportive Data

Fifty-two positive patient specimens were available for accuracy studies and correlated with results of organism-specific polymerase chain reaction (PCR). In addition, 20 negative samples from previous PCR testing were evaluated in verification. Using criteria established in verification, overall sensitivity of the assay is 82.7%, and specificity is 100% compared to single-analyte PCR tests. Sensitivity was lower due to the suboptimal recovery of *Borrelia burgdorferi* and increased to 97.4% without *B burgdorferi* included. A comment is added to reports that will reflect the limitations of detecting this organism.

The limit of detection was determined using four different organisms (*Enterococcus gallinarum, Pseudomonas aeruginosa, Leptospira interrogans,* and *Anaplasma phagocytophilum*) with an average sensitivity of 27 colony forming units per mL of whole blood for *E gallinarum* and *P aeruginosa,* and 63 copies per mL of whole blood for *L interrogans and A phagocytophilum*.

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 sequencing 56 samples representing diverse types of bacteria (including tickborne bacteria). All bacteria were detected and correctly identified by next-generation sequencing.

Clinical Reference

Kingry L, Sheldon S, Oatman S, et al. Targeted metagenomics for clinical detection and discovery of bacterial tick-borne pathogens. J Clin Microbiol. 2020;58(11):e00147-20. doi:10.1128/JCM.00147-20

Performance

Method Description

The test utilizes DNA extraction from EDTA whole blood and polymerase chain reaction (PCR) of a highly variable fragment of the 16S ribosomal RNA (rRNA) gene. If present, the amplified DNA is sequenced to obtain identification of the source organism from the patient sample. Specimens undergo total nucleic acid extraction, and the purified eluate is produced by the MP96 (Roche Diagnostics). The DNA eluate is concentrated two-fold from the specimen input volume.

PCR is performed on a LightCycler 480II instrument (Roche Diagnostics) to amplify approximately 530 base pairs (bp) of the bacterial 16S rRNA gene (V1-V3, exact length varies by species) with SYBR Green DNA detection.

Amplification inhibition is detected with a second PCR reaction performed using the extracted specimen spiked with a low concentration of positive control DNA. Due to high crossing point values reflecting the low abundance of pathogens, all patient samples for this test will undergo next-generation sequencing (NGS).

Samples are sequenced using NGS on a MiSeq sequencing platform (Illumina). The NGS process generates 500 bases of sequencing reads (250 bp in the forward and reverse directions). Due to the size of amplicon generated, there is a gap in coverage between forward and reverse sequences. Verification results and in silico analysis have demonstrated that forward sequence results (> or =210 bp) offer the same bacterial identity as full-length amplicon sequence for all organisms that can be reported by this assay. Quality filtering and results generation from NGS files is performed with Pathogenomix cloud-based software (RipSeq NGS).

High-quality sequence (Q > or =30) of 210 bp or more in length are sorted into clusters based on the observed sequence and used for identification. Positive and negative controls are used throughout all processes to ensure assay performance. Forward and reverse sequences are analyzed to determine bacterial identity using the cloud-based analysis program RipSeq NGS (Pathogenomix).(Rodino KG, Wolf MJ, Sheldon S, et al. Detection of tick-borne bacteria from whole blood using 16S ribosomal RNA gene PCR followed by next-generation sequencing. J Clin Microbiol. 2021;59(5):e03129-20. doi:10.1128/JCM.03129-20)

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
14 to 28 days

Specimen Retention Time
7 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.

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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-Specimen Identification by PCR (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
BRBST	Tickborne Bacterial PCR+Sequence, B	76575-0

Result ID	Test Result Name	Result LOINC® Value
BRBST	Tickborne Bacterial PCR+Sequence, B	76575-0