MAYO CLINIC LABORATORIES

# Test Definition: KPLA

Karius Spectrum, Plasma

## Overview

### Useful For

Rapid and minimally invasive detection of deep-seated and difficult-to-diagnose systemic infections throughout the body

Provide antimicrobial resistance detection for microbes known to utilize the antimicrobial resistance mechanism

### Testing Algorithm

For diagnostic application information see the following:
Karius Spectrum Diagnostic Application Pneumonia
Karius Spectrum: Diagnostic Application Endocarditis
Karius Spectrum: Diagnostic Application Febrile Neutropenia
Karius Spectrum: Diagnostic Application Invasive Fungal Infection
Karius Spectrum: Diagnostic Application Fever of Unknown Origin (FUO)

### Highlights

Karius Spectrum blood test leverages metagenomic sequencing of microbial cell-fee DNA to detect over 1000 fungi, bacteria, DNA viruses, and parasites using next-generation sequencing.(1) Absolute concentrations for each microbe are reported as molecules/100 nanoliters.

In addition to detecting over 1000 pathogens from blood, the Karius Spectrum test can detect common antimicrobial resistance (AMR) markers for 4 classes of antimicrobials across 18 bacterial pathogens (SCC*mec*, *mecA*, *mecC*, *vanA*, *vanB*, *bla*CTX-M and *bla*KPC).

For more information, see the following:
Karius Spectrum Liquid Biopsy for Infectious Diseases
In the Guidelines: Plasma Metagenomic Sequencing (MNGS) of Microbial Cell-Free DNA (mcfDNA)

### Method Name

Metagenomic Sequencing

### NY State Available

Yes

## Specimen

### Specimen Type

Plasma

## Specimen Required

**Collection Container/Tube:**
**Preferred:** Plasma preparation tube (PPT)
**Acceptable:** Lavender top (K2 EDTA)
**Submission Container/Tube:**
**Preferred**: PPT
**Acceptable**: Sterile polypropylene tube
**Specimen Volume**: 1 mL Plasma
**Collection Instructions :**
1. Gently invert tube 8 to 10 times to mix whole blood.
2. Centrifuge specimen as follows:
a. For PPT: Within 6 hours of collection, centrifuge at 1100 x *g* for 10 minutes.
b. For K2 EDTA:
i. Within 24 hours of collection, centrifuge at 1600 x *g* for 10 minutes. For tubes less than 4 mL, refer to tube manufacturer's instructions for centrifugation speed and time.
ii. Aliquot 1 mL of plasma into a sterile polypropylene tube, taking care not to disturb the buffy coat.
3. For complete instructions see Karius Spectrum Specimen Collection and Preparation Process.

## Specimen Minimum Volume

Plasma: 0.7 mL

## Reject Due To

| | |
|---|---|
| Incomplete or improper plasma separation | Reject |

## Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Plasma | Frozen (preferred) | 180 days | |
| | Ambient | 4 days | |

## Clinical & Interpretive

### Clinical Information

Karius Spectrum is a blood test leveraging metagenomic sequencing of microbial cell-fee DNA to detect over 1000 bacteria, fungi, parasites, and DNA viruses associated with deep-seated and difficult-to-diagnose systemic infections. The Karius Spectrum test may assist clinicians in reducing low-yield, sequential or diagnostic tests requiring tissue or fluid collection, which can delay treatment for vulnerable hospitalized patients.

In addition, Karius Spectrum provides antimicrobial resistance detection when appropriate. This is designed to help healthcare professionals use targeted therapy to improve patient outcomes. Four classes of antimicrobial resistance are

detected (methicillin-resistant *Staphylococci* [SCC*mec*, *mecA*, *mecC*], vancomycin-resistant *Enterococci* [*vanA*, *vanB*], carbapenem-resistant gram-positive bacteria [KPC], and extended spectrum beta-lactamase producing gram-negative bacteria [CTX-M]) for 18 bacterial pathogens.

For more information see Karius Spectrum In the Guidelines: Plasma Metagenomic Sequencing (MNGS) of Microbial Cell-Free DNA (MCFDNA).

## Reference Values

An interpretive report will be provided.

## Interpretation

A positive result indicates that microbial cell-fee DNA (mcfDNA) of one or more potentially pathogenic microorganisms was detected. The concentration of the mcfDNA detected will be provided in the report.

A negative result indicates absence of detectable mcfDNA from potentially pathogenic bacteria, fungi, parasites, and DNA viruses. A negative result does not rule the presence of a pathogen due to lack of a reference sequence in the database used or the presence of mcfDNA in quantities lower than the assay's limit of detection. Results should be interpreted in the clinical context of the patient.

This test detects antimicrobial resistance conferred by the following markers: SCC*mec*, *mecA*, *mecC*, *vanA*, *vanB*, CTX-M, or KPC. Evaluation for these markers will be performed when a microbe known to utilize the antimicrobial resistance mechanism is reported.

For more information see Understanding the Karius Spectrum Test Report.

## Cautions

The Karius Spectrum test does not detect RNA viruses.

Recent treatment with defibrotide sodium, an oligonucleotide drug derived from porcine tissue, may result in detectable microbial cell-free DNA from porcine-associated microbes and should be considered when interpreting results.

This test has been validated only for human plasma collected in EDTA anticoagulant.

Reliable results are dependent on adequate specimen collection, processing, transport, and storage procedures.

This test will report uncertain or unresolved species within the corresponding genus, eg, *Aspergillus flavus/oryzae* or *Neisseria* species.

The antimicrobial resistance marker may not always be linked with the microbe indicated.

The presence or absence of an antimicrobial resistance marker does not always correlate to the expected phenotype.

The assay analytical sensitivity is influenced by the depth of sequencing achieved. A minimum sequencing depth is required to pass quality control. Many batches achieve greater than this minimum sequencing depth resulting in enhanced sensitivity.

Concentration values for different microbes may not be comparable to each other.

To increase the clarity of the report as it relates to infections, microbes detected as frequently co-occurring are not reported when found together in one specimen. This may reduce the sensitivity to detect polymicrobial events such as mucosal membrane barrier disruptions, skin disruptions, gut injuries or aspiration pneumonia.

Microbes within a taxonomic family may not be reported when detected at less than 25% of the most abundant microbe within the corresponding taxonomic family.

Microbes within a taxonomic superkingdom are not reported when detected at less than 3% of the most abundant microbe within the superkingdom.

False-positive or false-negative results may occur for reasons including but not limited to sporadic contamination from specimen collection, reagent, and materials or hospital and laboratory environments, technical and biological factors.

The report of a microbe signifies the presence of its cell-free DNA in the patient's plasma specimen. It may or may not be the cause of an infection. Results should be interpreted within the context of clinical data, including medical history, physical findings, epidemiological factors, and other laboratory data.

### Supportive Data

Metagenomic sequencing was clinically validated in the SEP-SEQ Study, which enrolled 350 patients with suspected sepsis in the Emergency Department at Stanford University Hospital. The study demonstrated high agreement with standard diagnostics, increased pathogen detection compared to blood cultures and other microbiological tests, and potentially faster identification of the etiology of sepsis than the standard diagnostic workup.(1)

For more information see the following:
Karius Spectrum Clinical Evidence Guide
Karius Spectrum Invasive Fungal Infection Clinical Evidence Guide

### Clinical Reference

1. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. Nat Microbiol. 2019;4(4):663-674. doi:10.1038/s41564-018-0349-6
2. KC, Pfaller MA, eds. Manual of Clinical Microbiology. 12th ed. ASM Press; 2019
3. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 9th ed. Elsevier; 2019

## Performance

### Method Description

Karius Spectrum utilizes metagenomic sequencing of microbial-cell free DNA (mcfDNA) in plasma. The daily lab workflow involves preparing reagents and setting up automation systems for processing blood samples. Quality control materials are added to plasma samples to monitor issues such as contamination, technical errors, or sample mix-ups. DNA sequencing is performed directly from plasma without needing to extract DNA first, using a process tailored to recover microbial DNA efficiently. Each batch of samples is prepared together and sequenced using high-throughput instruments. Sequencing results are automatically analyzed and checked against background signals in real time. To

improve the accuracy of clinical results, advanced bioinformatics filters are used, which help distinguish between true infections and background microbes commonly found in healthy individuals or due to environmental contamination. The filters are based on large reference datasets and known microbial patterns. Sequencing reads are matched to a comprehensive microbial reference database, and results are filtered to ensure they meet quality standards. The clinical reportable range includes over 1000 bacteria, DNA viruses, fungi and parasites known to be associated with human infections.

For a listing of pathogens see https://kariusdx.com/our-solution/pathogens?product=spectrum.

### PDF Report
Referral

### Day(s) Performed
Monday through Saturday

### Report Available
2 to 6 days

### Specimen Retention Time
90 days

### Performing Laboratory Location
Karius Laboratory

## 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
The Karius Spectrum test was developed and its performance characteristics determined by Karius. The Karius laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) and is accredited by the College of American Pathologists (CAP) to perform high-complexity clinical laboratory testing. This test has not been reviewed or cleared by the US Food and Drug Administration.

### CPT Code Information
0152U

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| KPLA | Karius Spectrum, P | Not Provided |

# Test Definition: KPLA

Karius Spectrum, Plasma

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| KPLA | Karius Spectrum, P | Not Provided |