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Detection of Microbes Using Plasma Microbial Cell-Free DNA Metagenomic Sequencing

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Invasive diagnostic procedures can cause complications and adverse events

	Diagnostic Yield	Complication Rate	Adverse Events
Bronchoscopy	20-96%[1-6]	4-50%[1-6]	Bleeding, pneumothorax, hypoxia[1-6]
Lung Biopsy	60-80%[5,13,15,16]	25-35%[5,13,15,16]	Bleeding, pneumothorax, hemoptysis[5,13,15,16]
Brain Biopsy	80-99%[17,18]	Up to 12% morbidity 3.5% mortality[17,18]	Intracranial hemorrhage, edema, seizures[17,18]
Kidney Biopsy	80%-85%[8,14]	~8%[8,14]	Bleeding, AVF formation, Infection[8,14]
Joint Aspiration / Bone Biopsy	50-86% (hip); 88-96% (knee)[10,11,12]	0.05%-16%[10,11,12]	Infection, cartilage damage, bleeding[10,11,12]
Lumbar Puncture	16-48%[7,9]	6-25%[7,9]	Headache, infection, bleeding, cerebral herniation[7,9]

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2. Kamel T et al. *Intensive Care Med.* 2020;46(3):463-474
3. Bauer PR et al. *Eur Respir J.* 2019;54(1)
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7. Nothem M et al. Chest Annual Meeting 2020, October 18-21, 2020
8. Cooper S et al. . *American Journal of Roentgenology.* 2019;212(3):570-575
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14. Luciano RL et al. *Am J Kidney Dis.* 2019;73(3):404-415
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16. Sobrino JA et al. *J Surg Res.* 2019;236:106-109
17. Mathon B et al. *Eur J Intern Med.* 2020;80:78-85
18. Yong RL et al. *World Neurosurg.* 2013;79(1):53-54

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Targeted antimicrobial therapy is imperative to improve outcomes

26% of patients on inappropriate antibiotics will be readmitted to the hospital within 30 days.[1]

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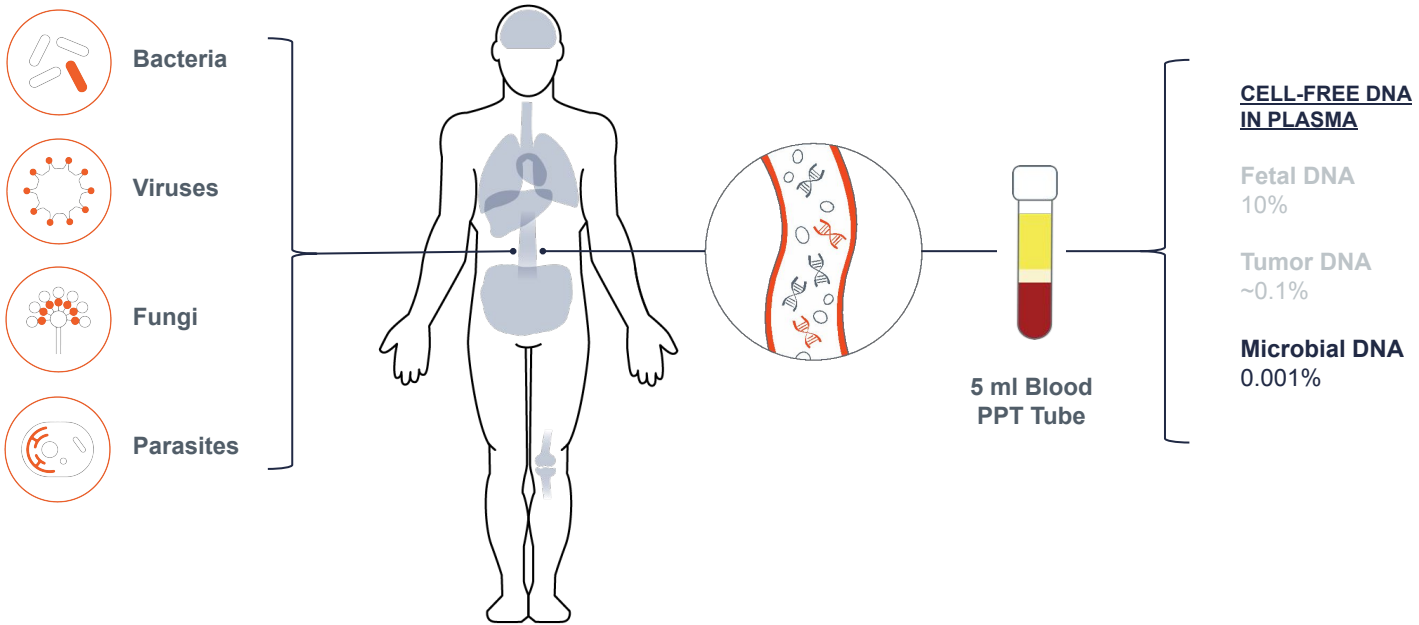
***patients with resistant gram-negative rod infections**

1. Zilberberg MD et al. *Antimicrob Resist Infect Control*. 2017;6:124

Microbial cell-free DNA (mcfDNA)



1. Blauwkamp, T. A. et al. *Nat.Microbiol*. 4, 663–674 (2019)
2. Hu, P., Liang, D., Chen, Y. et al. J. *TranslMed*. 17, 124 (2019)
3. Volik, S. et al. *Mol. Cancer Res*. 14, 898–908 (2016)

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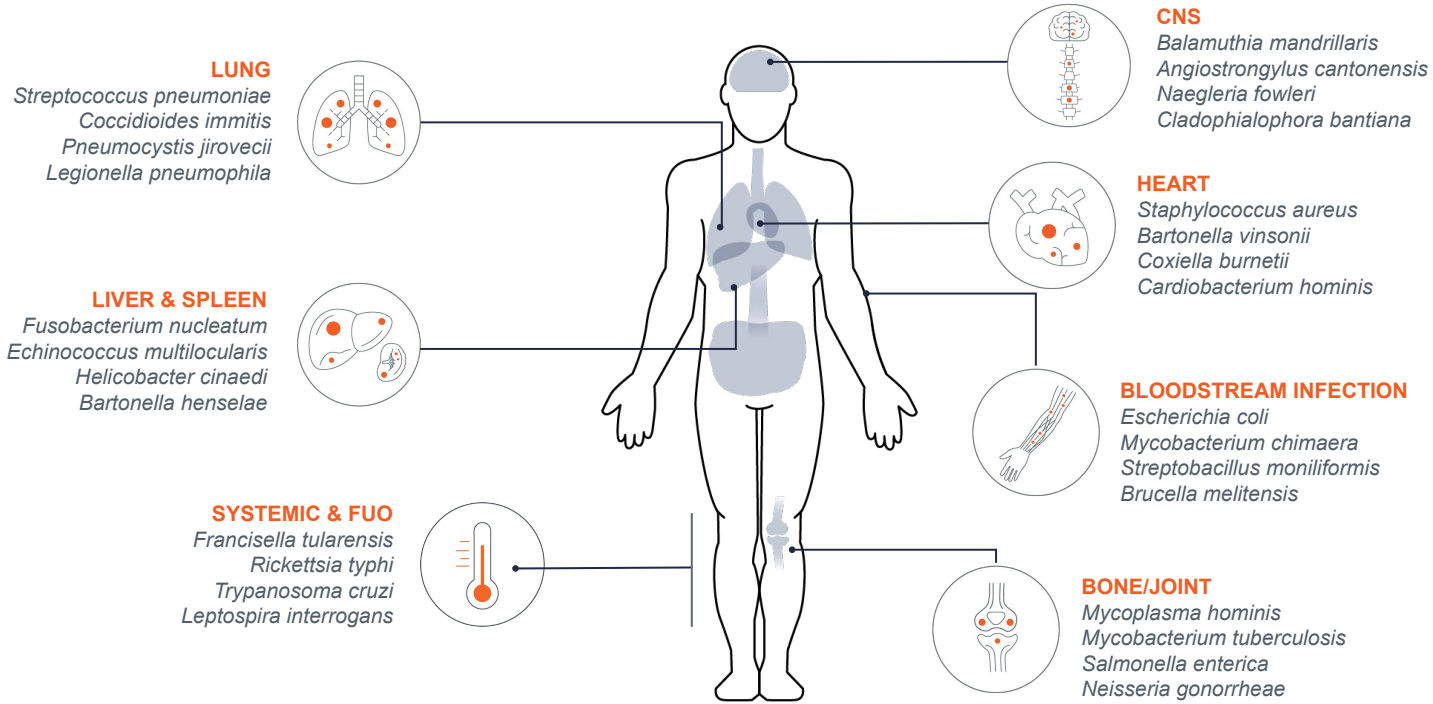
Comparison with other diagnostics

	Plasma mcfDNA Metagenomic Sequencing	Culture	Antigen Tests	Serology	PCR	Other NGS
Advantages	• Plasma only specimen • Broad detection >1,000 pathogens • Multiple pathogen types (DNA viruses, bacteria, fungi, parasites)	• Traditional “gold standard” • Can isolate colony • Can perform susceptibility	• Rapid • Inexpensive	• May detect non-culturable organisms	• More sensitive • More specific • Shorter TAT • Multiplex PCR panels 4 to >25 pathogens	• Higher genomic coverage
Limitations	• Send-out model adds 24hrs • No RNA pathogens	• Long turnaround time • Limited sensitivity • Limited species resolution • May need invasive sample	• Variable Sensitivity • Variable Specificity	• Limited utility in IC hosts • Subject to cross-reactivity • Weeks to months for antibody development • Prone to FP and/or persistent positivity	• Few pathogens • Require prior knowledge of suspected organism • May require send-out • Target erosion	• Sample of infected tissue required • Send-out model adds 24hrs • Mostly research-grade

Plasma mcfDNA Metagenomic Sequencing non-invasively detects deep-seated and difficult to diagnose systemic infections



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AMR Detection

Drug-resistant infections threaten millions of people each year, including hospitalized and other high-risk patients

>2.8M people are sickened by drug-resistant infections annually in the United States, with 35,000 deaths[1]

~70% of pathogens causing bacterial infection in hospitals are resistant to at least one prevalently used antibiotic in the United States[2]

~27% of pathogens causing infections after chemotherapy are estimated to be resistant to standard prophylactic antibiotics in the United States[3]

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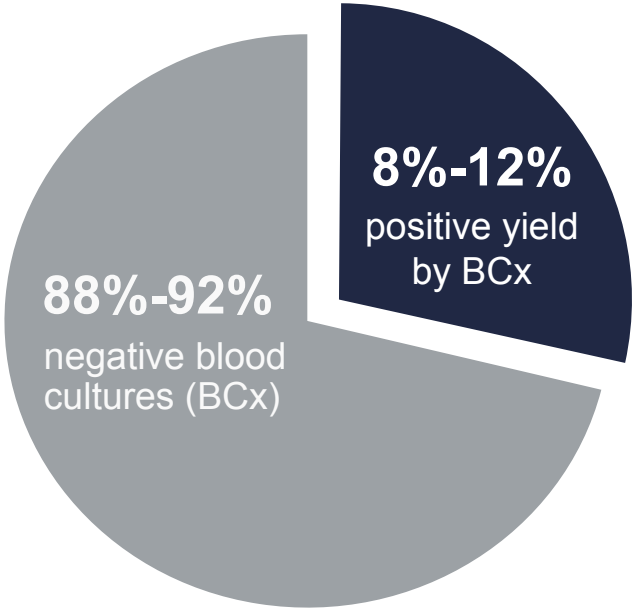
1. CDC: https://www.cdc.gov/drugresistance/biggest-threats.html
2. FDA: https://www.fda.gov/drugs/information-consumers-and-patients-drugs/battle-bugs-fighting-antibiotic-resistance
3. Teillant, A et al. *Lancet Infect Dis.* 2015 Dec;15(12):1429-37. Epub 2015 Oct 22.

Ability to target therapy is often limited by culture-dependent methods with low yield in hospitalized patients



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Source: Melvin P. Weinstein, and L. Barth Reller, 2019. Laboratory Detection of Bacteremia and Fungemia, p.28. In: Carroll KC, Pfaller MA Manual of Clinical Microbiology, 12th Edition. ASM Press, Washington, DC

Low yield contributes to the use of empiric therapy and diagnostic delays

FACTORS IMPACTING AMR

Low yield with SOC diagnostics

Use of inappropriate **empiric therapy**

Sequential testing and **diagnostic delays**

UNMET NEED: There is a need for diagnostic tools that can both rapidly detect pathogens **and** associated antimicrobial resistance to help clinicians effectively target treatment and improve patient outcomes

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Source: Antimicrobial Resistance Collaborators. *Lancet*. 2022 12-18 February; 399(10325): 629–655. Published online 2022 Jan 19.

Plasma mcfDNA Metagenomic Sequencing can detect common AMR markers for 4 classes of antimicrobials across 18 bacterial pathogens

Gram-Positive Bacteria	AMR Marker						
	SCC*mec*	*mecA*	*mecC*	*vanA*	*vanB*	CTX-M	KPC
Methicillin-resistant *Staphylococci*							
S. aureus	+	+	+				
S. epidermidis		+	+				
S. lugdunensis		+	+				
Vancomycin-resistant *Enterococci*							
E. faecalis				+	+		
E. faecium				+	+		

Gram-Negative Bacteria	AMR Marker						
	SCC*mec*	*mecA*	*mecC*	*vanA*	*vanB*	CTX-M	KPC
Carbapenem resistant and extended spectrum beta-lactamase (ESBL) producing bacteria							
E. cloacae						+	+
E. coli						+	+
K. aerogenes						+	+
K. pneumoniae						+	+
K. oxytoca						+	+
P. mirabilis						+	+
P. vulgaris						+	+
S. bongori						+	+
S. enterica						+	+
S. marcescens						+	+
P. aeruginosa						+	+
A. baumannii						+	+
A. calcoaceticus						+	+

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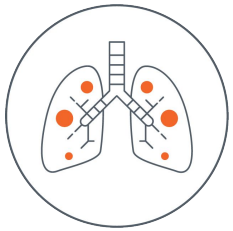
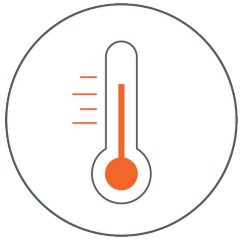
AMR markers will be reported alongside microorganism detection

Diagnostic applications of Plasma mcfDNA Metagenomic Sequencing



Complicated Pneumonia

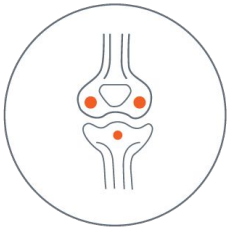
Invasive Fungal Infections



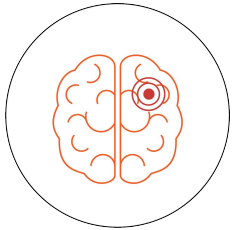
FUO

Endocarditis

Febrile Neutropenia



Osteomyelitis



Focal Abscess

Zoonoses

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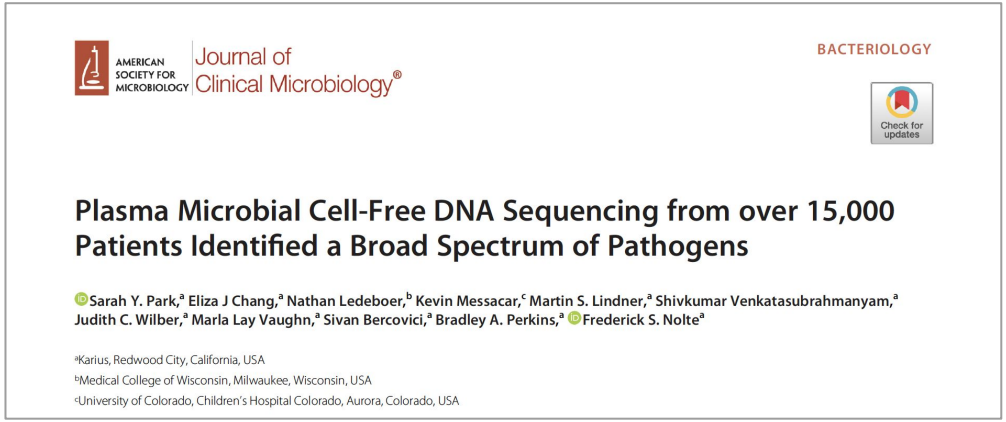
Clinical Studies

Plasma microbial cell-free DNA sequencing from over 15,000 patients identified a broad spectrum of pathogens

- Retrospective study of large cohort of patients in which microbial cell-free DNA (mcfDNA) was identified and quantified
- The testing cohort included 15,165 patients from both adult and pediatric populations (65% vs 35% respectively)



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Plasma Microbial Cell-Free DNA Sequencing from over 15,000 Patients Identified a Broad Spectrum of Pathogens

Sarah Y. Park,[a] Eliza J Chang,[a] Nathan Ledeboer,[b] Kevin Messacar,[c] Martin S. Lindner,[a] Shivkumar Venkatasubrahmanyam,[a] Judith C. Wilber,[a] Marla Lay Vaughn,[a] Sivan Bercovici,[a] Bradley A. Perkins,[a] Frederick S. Nolte[a]

[a]Karius, Redwood City, California, USA
[b]Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[c]University of Colorado, Children's Hospital Colorado, Aurora, Colorado, USA

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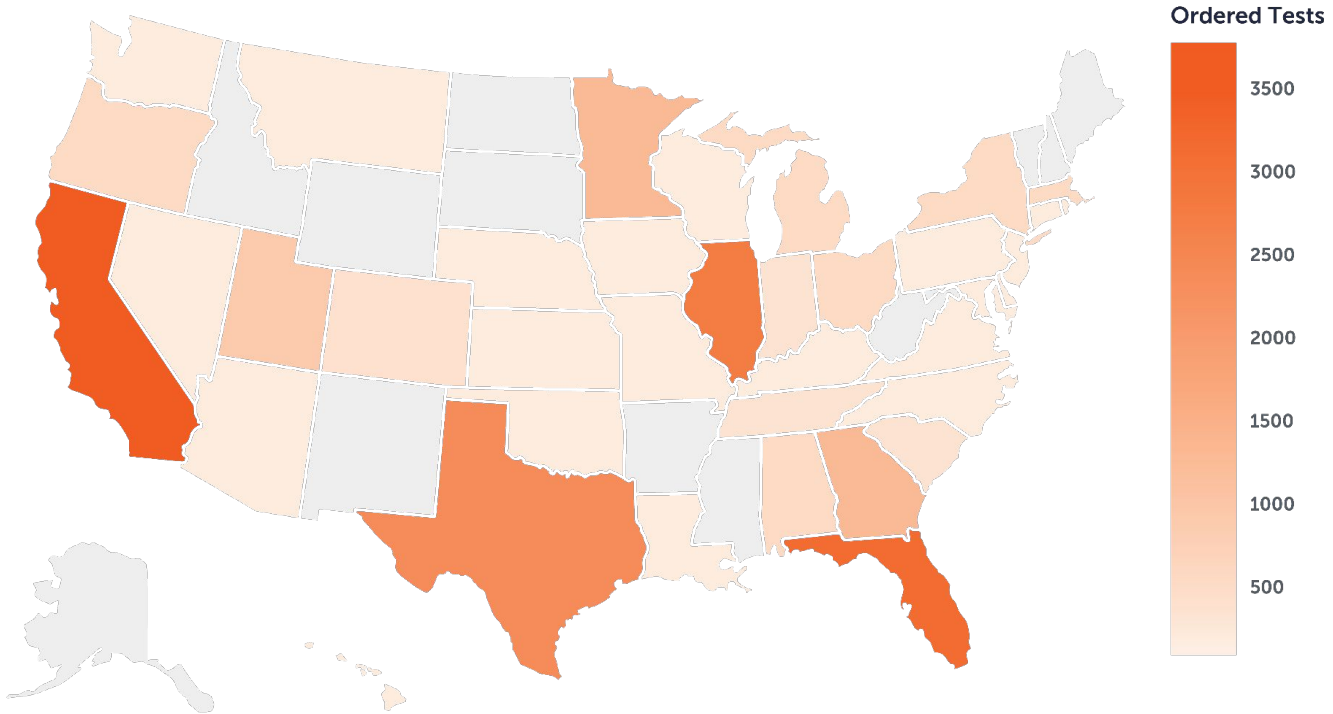
Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

The study includes 15,165 patients from 39 states and the District of Columbia



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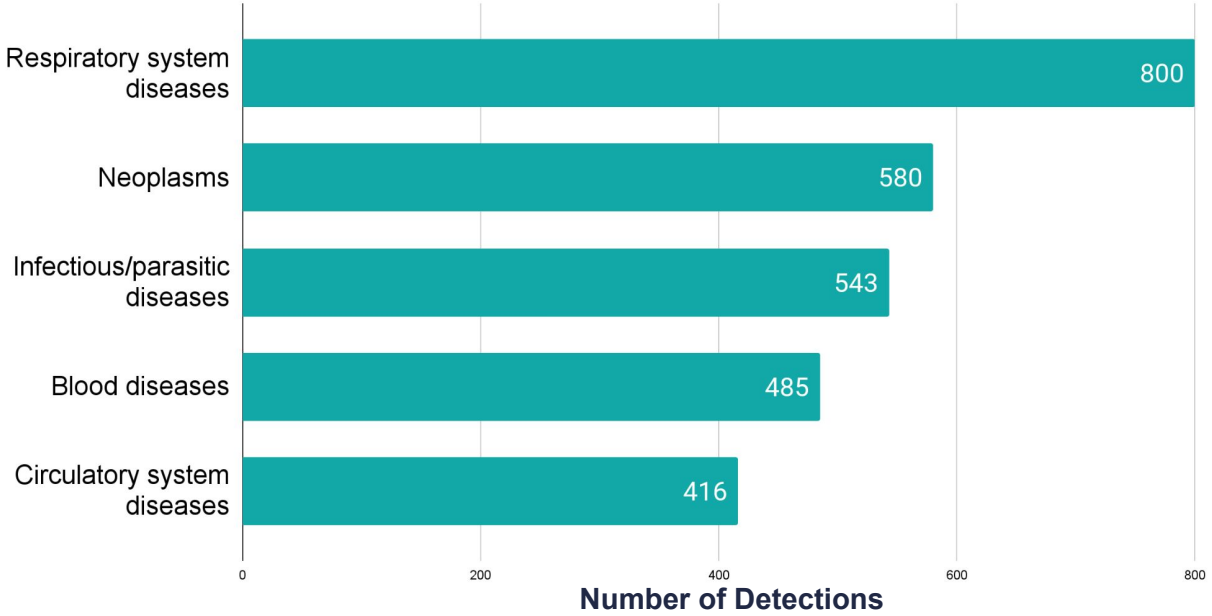
Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Application across a variety of clinical scenarios

TOP 5 CLASSIFIED ICD-10 DIAGNOSIS CODES SUBMITTED*



For remaining classifications, view full table in publication

** Of instances where an ICD-10 diagnosis code was submitted and mappable; a diagnosis code was provided in 29% of cases*

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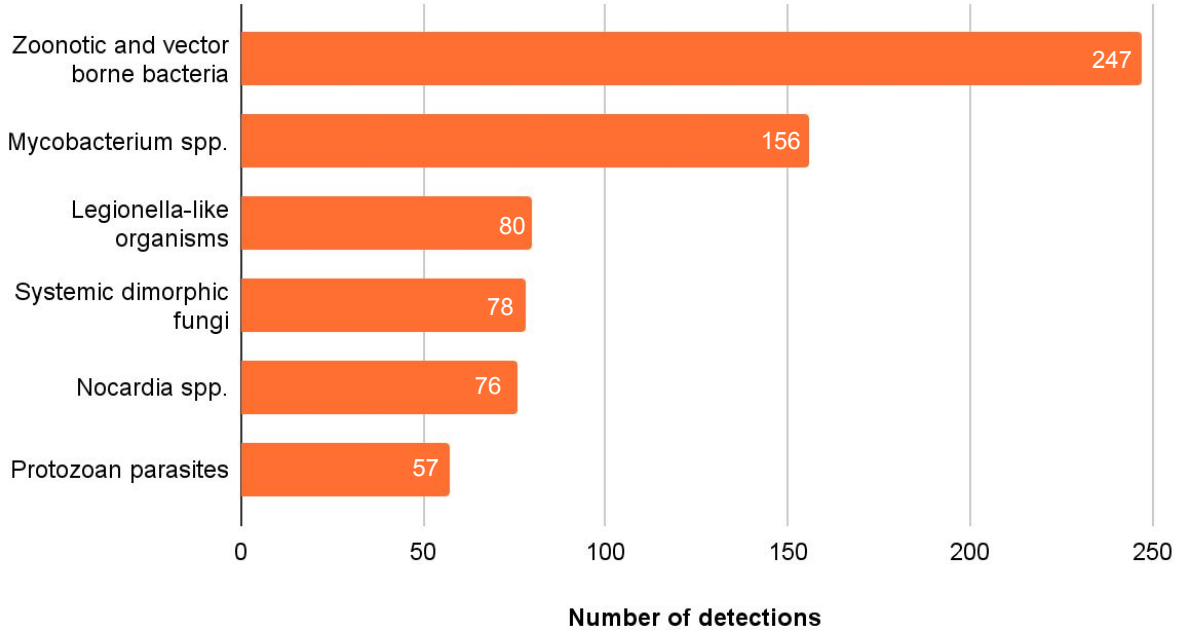
Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Across a large cohort of patients, plasma mcfDNA metagenomic sequencing detected diagnostically challenging pathogens (n = 683)



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Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Moveover, plasma mcfDNA metagenomic sequencing identified pathogens across a range of fungal, bacterial, and viral species

The 50 most commonly detected taxa included 36 bacteria, 9 viruses, and 5 fungi:

Species	Findings
 **Fungi**	Opportunistic pathogens detected including ***Aspergillus* spp.**, ***Pneumocystis jirovecii*, *Mucorales***
 **Bacteria**	Key detections of opportunistic pathogens including ***Mycobacterium* spp.**, ***Legionella* spp.**, ***Nocardia* spp**.
 **Viruses**	Among viruses detected, the **Human Herpesviruses** predominated with 81% of viral detections

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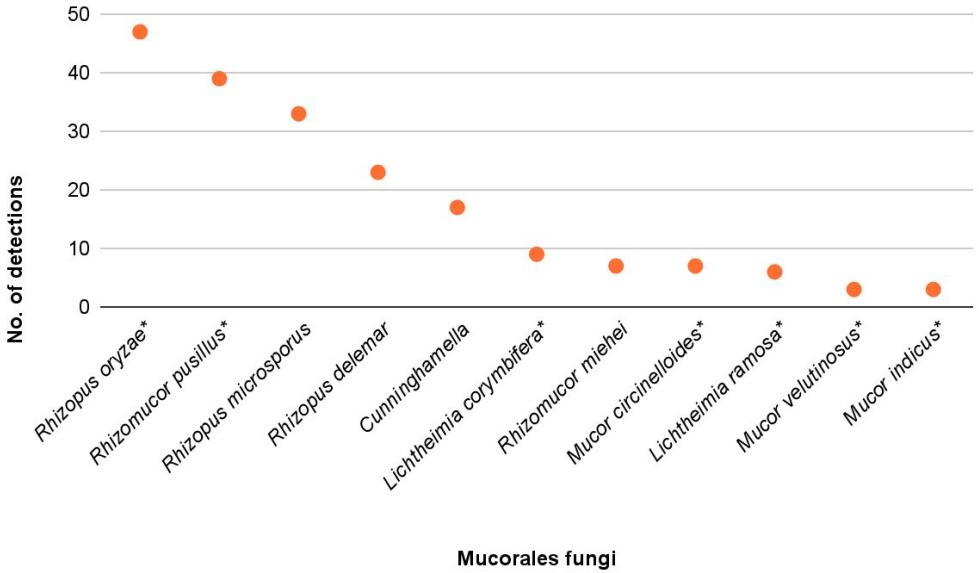
Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Mucorales represented 11% (n=196) of all fungal detections

Frequency distribution of detections in the order Mucorales, n=196 (11% of all fungal detections, N=1,776)

*indicates taxa commonly implicated in human mucormycosis

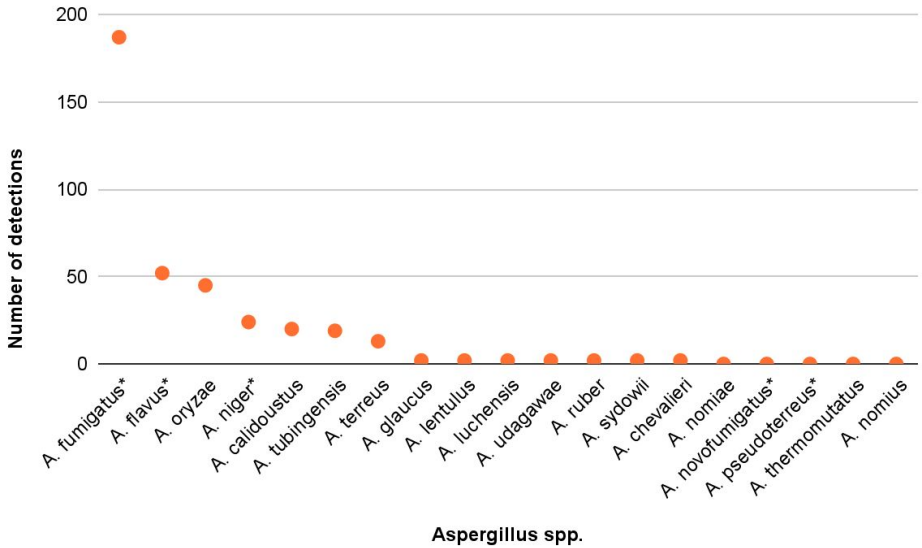


Park, SY et al. *J Clin Microbiol*. 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Aspergillus spp. represented 21% (n=374) of all fungal detections

Frequency distribution of *Aspergillus* spp. detections, n=374 (21% of all fungal detections, N=1,776).

A. fumigatus dominated.



* indicates most common pathogenic species.

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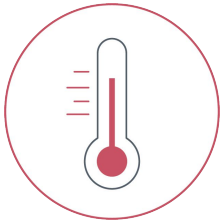
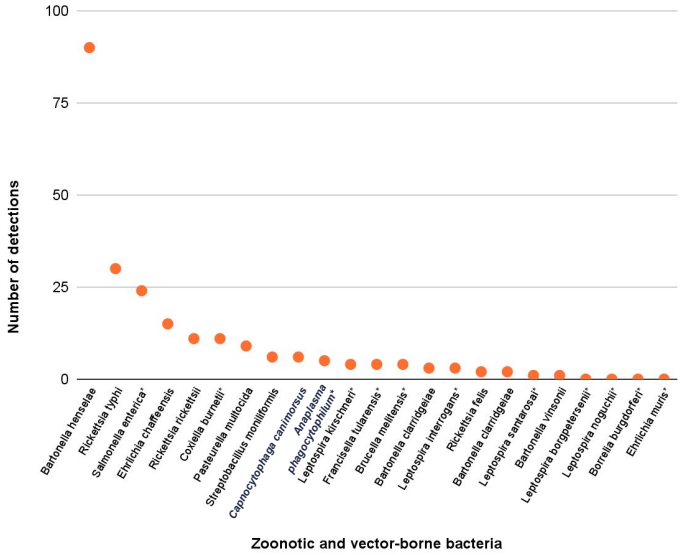
Park, SY et al. *J Clin Microbiol*. 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Plasma mcfDNA metagenomic sequencing identified pathogens causing zoonotic and vector-borne bacterial infections



- Diagnosis of this microbes is challenging due to non-specific symptoms and lack of sufficient data on standard of care tests, including serological and molecular tests that can have a long turnaround time
- plasma mcfDNA metagenomic sequencing detected 20+ pathogens causing zoonotic and vector-borne bacterial infections
- These detections include 12 bacteria causing a nationally notifiable infectious disease (demarcated by *)
- *B. henselae* (36%) was the most common pathogen identified in this category

Frequency distribution of zoonotic and vector borne bacteria detection (n=247)



Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Key Takeaways

- Plasma mcfDNA metagenomic sequencing:
  - has a rapid turnaround time, with median time from sample receipt to reported result of 26 hours
  - can detect diagnostically challenging microbes such as opportunistic and systemic dimorphic fungi and zoonotic and vector-borne pathogens
  - provides absolute quantification shown to correlate well with single-analyte qPCR measurements of DNA viruses
- Due to the breadth of detection, there is the potential to enhance disease surveillance with plasma mcfDNA metagenomic sequencing
- Utilizing appropriate diagnostic stewardship with timely use, expert guidance and interpretation of its results can optimize the application of plasma mcfDNA metagenomic sequencing.

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Park, SY et al. *J Clin Microbiol.* 2023 Aug 23;61(8):e0185522. doi: 10.1128/jcm.01855-22. Epub 2023 Jul 13.

Utility of serial microbial cell-free DNA sequencing for inpatient and outpatient pathogen surveillance among allogeneic hematopoietic stem cell transplant recipients

Fung M, Patel N, DeVoe C, Ryan C, McAdams S, Pamula M, Dwivedi A, Teraoka J, Smollin M, Sam S, Perkins B, Chin-Hong P. *Open Forum Infectious Dis.* 2024.

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Background

- Infections are a significant source of morbidity and mortality among hematopoietic stem cell transplantation (HCT) recipients[1]
  - Leading cause of early death +100 days post-HCT
- Causative pathogen recovered infrequently[2]
  - Approximately one third of immunocompromised patients with febrile neutropenia
- Limitations of standard microbiologic testing (SMT) methods for pathogen detection are[3-5]:
  - Low culture yield
  - Difficulty isolating fastidious organisms
  - Multiple tests impacting timing and cost

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1. Lindsay J, et al. *Transplant Cell Ther.* 2021 Sep;27(9):798.e1-798.e10. 2. Benamu E, et al. *Clin Infect Dis.* 2022;74(9):1659-1668. 3. Schlaberg R, et al. *Arch Pathol Lab Med.* 2017 Jun;141(6):776–86. 4. Vartoukian SR. *J Oral Biosci.* 2016 Nov;58(4):142–9. 5. Caliendo AM, et al. *Clin Infect Dis.* 2013 Dec;57(suppl3):S139-70.

Background (cont.)

- Plasma mcfDNA metagenomic sequencing may overcome some limitations of SMT
  - Pathogen agnostic
  - Each mcfDNA sequencing detection accompanied by molecules per microliter (MPM)
    - Quantitative measure of pathogen density which could aid in monitoring and earlier detection
- As a surveillance tool, mcfDNA sequencing could provide clinical utility and validity in HCT recipients in the inpatient and outpatient setting by:
  - Monitoring for CMV
  - Detection of pathogens relative to SMT
  - Detection of diagnostically-challenging pathogens not identified by SMT

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Study Objectives

- Assess the additive diagnostic performance of plasma mcfDNA sequencing at detecting CMV
  - Assess correlation between mNGS MPM and CMV viral load
- Determine if plasma mcfDNA sequencing could result in earlier detection of clinically relevant pathogens relative to SMT
- Identify the types of pathogens that could be detected using plasma mcfDNA sequencing when SMT is negative

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Study Design & Population

- Prospective, observational cohort study
- Adult HCT patients receiving care at the University of California San Francisco Health between 8/2016 and 5/2018
- Inclusion criteria:
  - Age ≥ 18 years
  - Receiving an allogeneic HCT, prior to start of their conditioning regimen
  - Willingness to submit to serial blood sample collections in inpatient/outpatient settings

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

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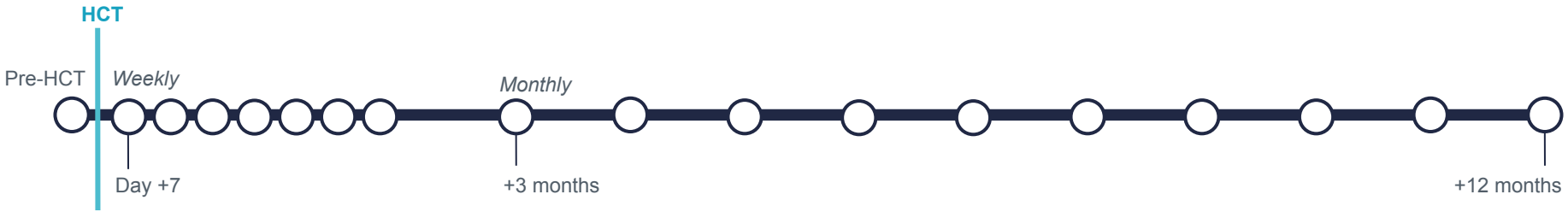
Methods: Sampling Schema

- Plasma mcfDNA sequencing samples
  - One blood sample before HCT
  - Weekly from day +7 to +63
  - Monthly starting at 3 months until study end (12 months post-HCT)
- Weekly CMV qPCR for individuals who were D+ or D-/R+
- All other pathogen detections were performed as standard of care using SMT based on clinical suspicion



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Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Methods: Identification of Infectious Events

- Potential infectious events were identified by discharge summaries in the electronic health record (EHR)
- Onset of infectious event was defined as either:
  - Date of positive SMT (e.g. culture)
  - First date of clinical signs/symptoms associated with an infectious event (e.g. fever)
  - Date of procedure diagnosing an infection (e.g. chest X-ray).
- For events with no causative pathogen identified by SMT, two infectious diseases clinicians reviewed the EHR to identify infection onset
  - Adjudicated all preceding plasma mcfDNA detections to determine if any could be potential causes of the infectious event

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Results: Plasma mcfDNA metagenomic sequencing demonstrates strong diagnostic performance for CMV vs qPCR

	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)	Overall Percent Agreement (OPA)
Overall	100%	88.9%	89.7%
D+/R+	100%	87.0%	88.9%
D+R-	*	100%	100%
D-/R+	100%	81.8%	82.6%
D-/R-	*	100%	100%
Inpatient	100%	82.6%	84.0%
Outpatient	100%	100%	100%

- PPA and NPA were calculated based on 1st patient encounter with mcfDNA and qPCR test drawn within 24 hours of one another.
- Test concordance was calculated as the total number of tests with dual agreement divided by total number of tests performed.

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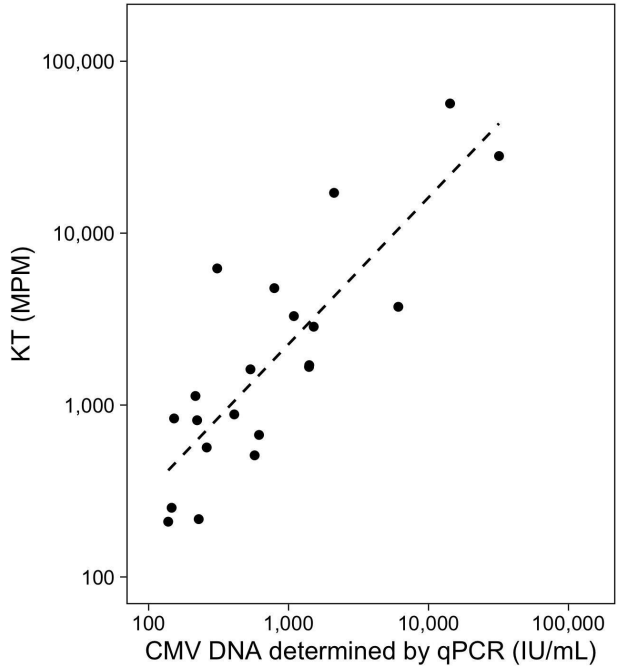
*Indicates that PPA was not computable because there were 0 CMV detections by both quantitative polymerase chain reaction and Karius Test®.

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Results: Correlation between plasma metagenomic sequencing and CMV viral load



- Strong correlation between plasma mcfDNA molecules per microliter (MPM) and CMV DNA determined by quantitative polymerase chain reaction (qPCR)
  - r^2: 0.84, $p<0.001$
- Identified an equation to convert between plasma mcfDNA MPM and CMV DNA
  - $Log_{10}[qPCR] = -0.254 + 0.927*log_{10}[MPM]$
  - CMV DNA: 137 IU/mL ~ mNGS MPM: 379
  - CMV DNA: 1000 IU/mL ~ mNGS MPM: 3238

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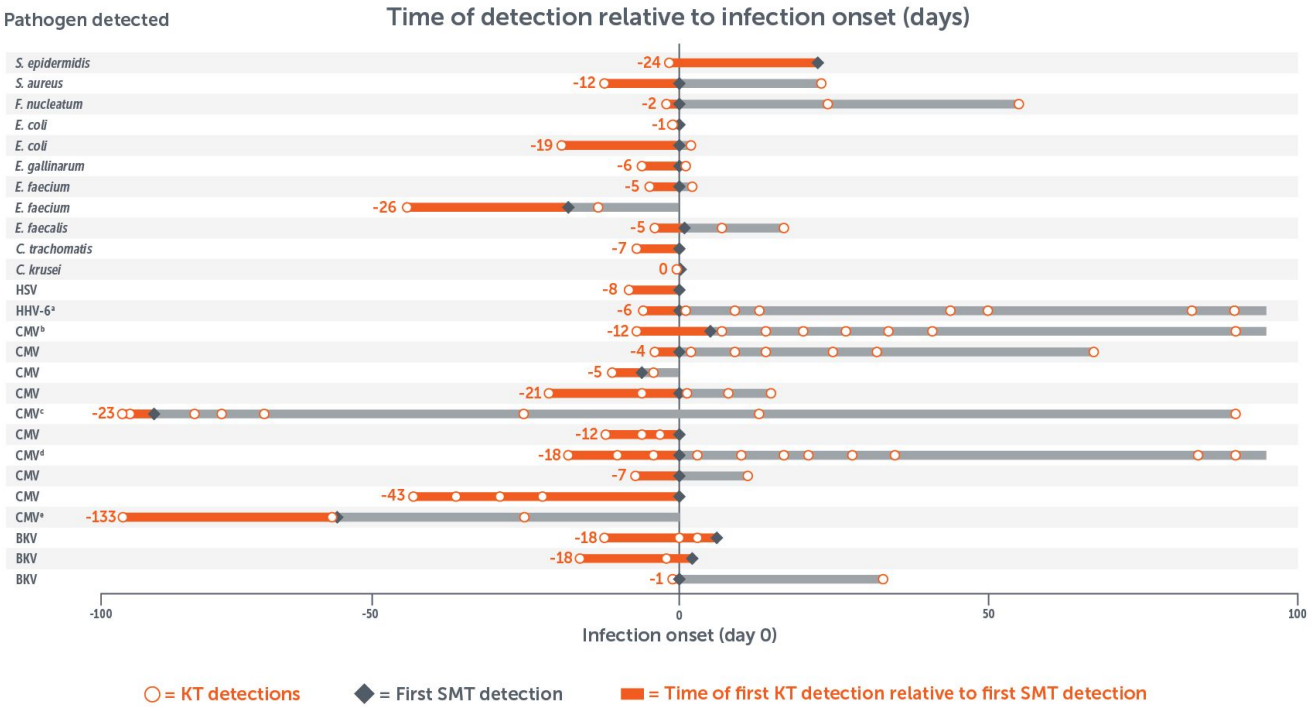
Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Results: Earlier detections by plasma metagenomic sequencing



- Of the 32 instances where both plasma mcfDNA and SMT detected pathogens, mcfDNA detections were earlier for 26/32 infectious events (81%)
  - Plasma mcfDNA detections were a median (IQR range) of **12 (23 to 5) days earlier** than SMT
- mcfDNA remained positive multiple times before/after SMT pathogen detection

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Footnotes: Infection onset is the date where individual presented with clinical signs/symptoms associated with infection or had a diagnosing procedure and adjudicated by two independent infectious diseases clinicians. a – continued to have positive KT detection on +153 days after infection onset b – continued to have positive KT detections on +99, +132, +162, +181 and +230 days after infection onset c – truncated scale on left; first KT detection was at day -108, followed by -102 and -94 days before SMT detection on day -85 before infection onset d – continued to have positive KT detection on +113 days after infection onset e – truncated scale on left; first KT detection was at day -189 before SMT detection on day -56 preceding infection onset

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Results: Additive diagnostic value of plasma mcfDNA metagenomic sequencing

Pathogen	Infection type		**Hospital Length of stay**	**Days between death and infection onset**
Bordetella hinzii	Pneumonia	100	11	11
Mycoplasma hominis	Pneumonia	18	29	Alive at 12 months post-HCT
Human mastadenovirus	Ileitis with diarrhea	155	2	Alive at 12 months post-HCT
E. coli *E. faecium*	Peritonitis/intraperitoneal abscess	37	57	29
HSV-2	Lesion on mons pubis	21	47	Alive at 12 months post-HCT

- mcfDNA was able to identify 6 pathogens in 5 cases when SMT was negative
  - mcfDNA detected fastidious pathogens (*Bordetella hinzii* and *Mycoplasma hominis*)
  - mcfDNA detected pathogens in infection types (pneumonia and peritonitis), where diagnosis typically requires an invasive procedure

KARIUS

Fung M, et al. *Open Forum Infect Dis.* Published online July 3, 2024:ofae330.

Conclusion

- This study is the first to show how the plasma mcfDNA may be a useful surveillance tool for infection among HCT recipients in the inpatient and outpatient settings
- Key takeaways:
  - Plasma mcfDNA MPM and quantitative CMV DNA were strongly correlated (r^2: 0.84, *p<0.001*)
  - Plasma mcfDNA detected pathogens several days earlier than SMT (median 12 days earlier)
  - Plasma mcfDNA identified 6 additional probable pathogens not detected by SMT, including fastidious organisms (*Bordetella hinzii* and *Mycoplasma hominis*).

KARIUS

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Karius Test

A Liquid Biopsy for Infectious Diseases

The Karius Test is a blood test based on metagenomic sequencing of **microbial cell-free DNA**.

1 blood sample[†]
(specifically plasma)

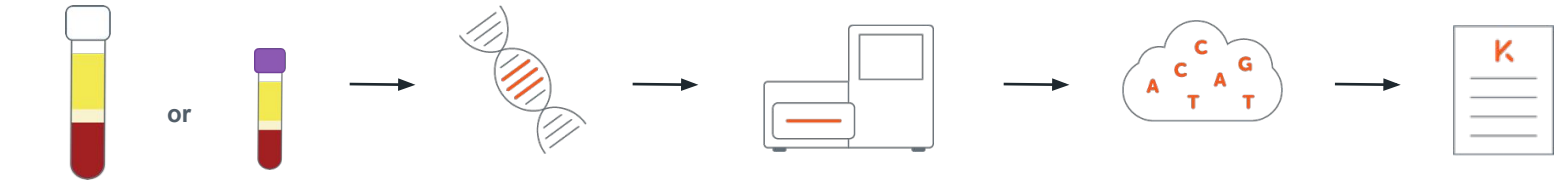
1 day processing[*]

1000+ detectable pathogens

[†] https://kariusdx.com/the-karius-test/test-process/.
* >85% of specimens received by 8:30 AM (PT) Monday through Saturday are reported the next day after sample receipt, with bacterial AMR marker detection available the following day.

Karius Test – Process

Reports are typically available one day after specimen receipt*



5 ml Blood PPT Tube† (specifically plasma)

2 ml Blood K2-EDTA

Specimen Processing

Sequencing

Analysis

Report

The Karius laboratory is **CLIA-certified** and **CAP-accredited** to perform high-complexity clinical laboratory testing.

* >85% of specimens received by 8:30 AM (PT) Monday through Saturday are reported the next day.
† https://kariusdx.com/the-karius-test/test-process

KARIUS

Karius Test Report

1 MICROBE DETECTIONS

Microbes are reported that meet the following: (1) part of the proprietary database, and (2) had cell-free DNA detected in the patient's plasma specimen in **statistically significant** amounts.

2 CLASSIFICATION OF MICROBES

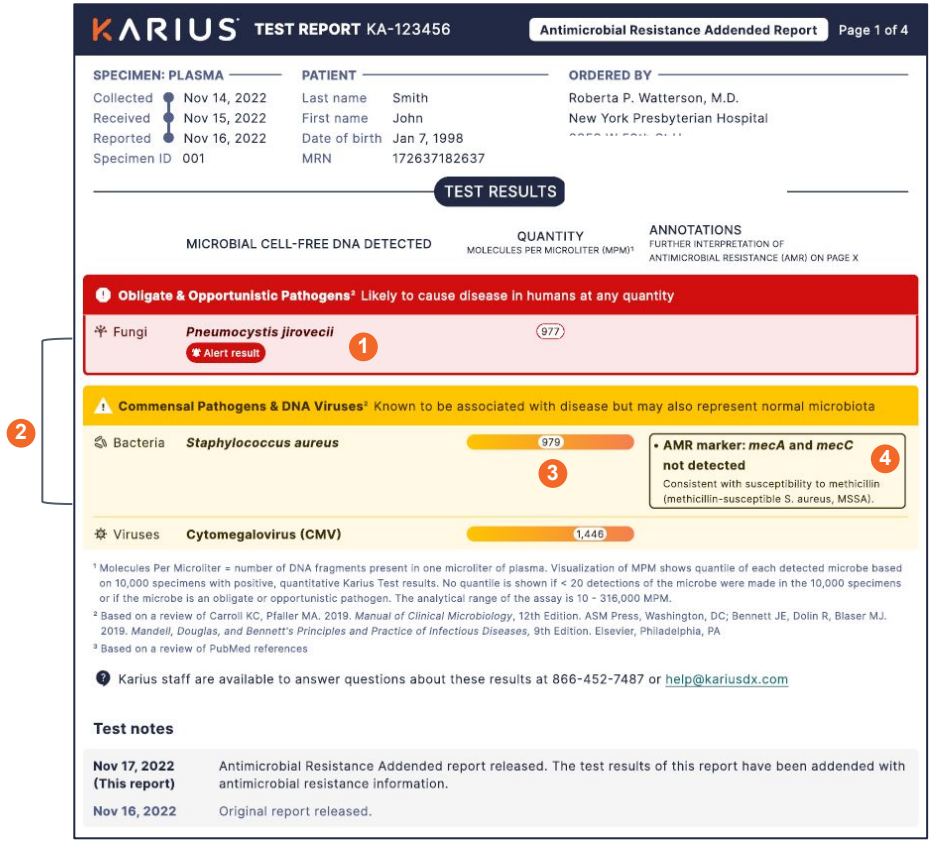
Microbes are classified into one of three categories: (1) Obligate & Opportunistic Pathogens, (2) Commensal Pathogens & DNA Viruses, or (3) Microbes with Published Case Reports.

3 QUANTIFICATION

The concentration of the microbial cell-free DNA is measured in molecules of DNA fragments per microliter of plasma (molecules per microliter, MPM). Gradient visualization of MPM quantile based on 10,000 specimens with positive Karius Test results reflects relative abundances. MPM value should not be compared across different microbes.

4 ANTIMICROBIAL RESISTANCE

The presence or absence of 7 AMR markers (SCCm*ec*, *mecA*, *mecC*, *vanA*, *vanB*, CTX-M, and KPC) are reported across 18 bacterial pathogens. Results are typically reported as an amended report the following day.



Accessing Karius Test Results

Fax

Automatically transmitted via electronic fax

Karius Online Portal

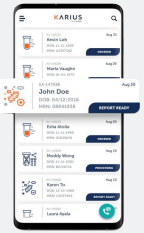
Same login for both portals

Karius Mobile App

Karius Mobile App

- Same login as desktop version
- Face or fingerprint authentication
- Available on App store and Google Play

- Real-time notifications
- Custom notification settings, for every step of the test process



- Easy to access test results
- Simple search (patient name, MRN, KA-ID)
- Quick and easy access to Customer Success

KARIUS

Karius Test®: Committed to Continuous Innovation

KEY MILESTONES

December 2016
Karius Test product launch: first samples analyzed with Karius Test

July 2020
Added 125 taxa to the list of reportable microbes

August 2021
Sensitivity improved for 183 high relevance taxa

February 2023
Sensitivity improved across the entire set of reportable microbes

September 2023
Expansion of AMR detection capability to 7 AMR markers (SCC*mec*, *mec*A, *mec*C, *van*A, *van*B, CTX-M, KPC) across 18 bacterial pathogens

May 2024
The Karius Test was granted designation as a Breakthrough Device from the FDA.

May 2018
Analytical platform launch (DC3) to identify and quantify microbes

May 2021
Sensitivity improved for 40 *Aspergillus spp.*

April 2022
Launched reporting of the SCC*mec* resistance markers consistent with methicillin-resistant (MRSA) or methicillin-sensitive (MSSA) *Staphylococcus aureus*

May 2023
Inclusion of Metagenomic Sequencing in 2023 Duke-ISCVID Criteria for Infective Endocarditis

December 2023
New report layout to facilitate the interpretation of results

KARIUS

More Information

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