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Microbial Cell-Free DNA Sequencing Diagnostics: A Critical Tool for Advancing Xenotransplantation Safety and Infection Monitoring

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Background & Rationale

Xenotransplantation offers a practical response to the chronic deficit of donor organs [1,2]. Its clinical **promise is tempered by xenosis**: hundreds of porcine microorganisms—including numerous viruses, bacteria, parasites, fungi—require surveillance [3,4]. As donor animals can be selected and quarantined weeks before procurement, source herds may be maintained under designated pathogen-free conditions and screened more rigorously than allograft donors. Recent International Xenotransplantation Association position guidance noted that **metagenomic cell-free DNA sequencing may supplement standard assays** for unknown pathogens [5], underscoring the value of unbiased agnostic sequencing alongside serology and culture in both animals and recipients. Here, we report the **analytical validation of an mcfDNA based test optimized for xenotransplantation** applications to detect porcine pathogens in plasma, including porcine plasma and human plasma following xenotransplantation.

What is microbial cell-free DNA?

Microbial cell-free DNA (mcfDNA) comprises short **genomic fragments shed into plasma** by microbes; untargeted next-generation sequencing of these fragments enables broad, culture-independent pathogen identification directly from blood [6]. As circulating mcfDNA may mirror organism burden across body sites, its quantification offers a rapid, minimally-invasive means to aid in the interpretation of the signal.

References

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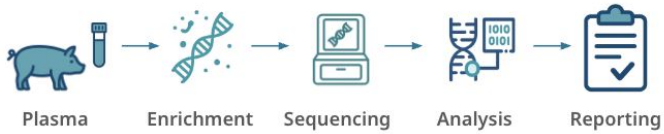
[3] Fishman, Jay A., and Nicolas J. Mueller. 2024. "Infectious Diseases and Clinical Xenotransplantation." Emerging Infectious Diseases 30 (7): 1311–18.
[4] Stewart, Adam G., and Jay A. Fishman. 2025. "Surveillance and Prevention of Infection in Clinical Xenotransplantation." Clinical Microbiology Reviews 38 (1).
[5] Hawthorne, Wayne J., et al. 2025. "International Xenotransplantation Association (IXA) Position Paper on the History, Current Status, and Regulation of Xenotransplantation." Transplantation, April.
[6] Blauwkamp, Timothy A., et al. 2019. "Analytical and Clinical Validation of a Microbial Cell-Free DNA Sequencing Test for Infectious Disease." Nature Microbiology 4 (4): 663–74.

Method



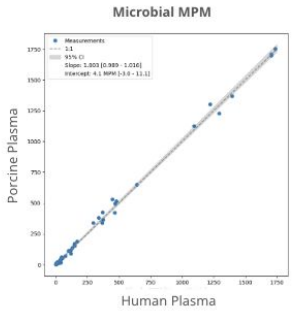
Cell-free DNA was extracted from 350 µL of plasma and enriched for microbial fragments through an unbiased proprietary laboratory process. Internal standards and controls were incorporated to verify sequencing depth and monitor for cross-contamination. Reads were filtered against the human and porcine genomes, then aligned to a curated database of over 20,000 microbial assemblies, reporting clinically relevant taxa. Analytical filters were applied to mitigate the impact of reagent contamination, incomplete databases, and genomic homology, ensuring high specificity. Detected microbes are reported alongside their estimated concentration in molecules per microliter (MPM).

Validation Methodology

The analytical performance was validated using a method-based approach (Blauwkamp et al., Nature Microbiology, 2019).

Contrived Samples		A panel of 13 representative microbes (P13) selected to capture a broad range of factors (e.g., GC content, genome size, and superkingdom) was used to characterize the analytical sensitivity of the assay on human background.
		The P13 representative panel was used to demonstrate equivalent measurement performance in human and porcine plasma.
In Silico Simulations		The analytical validation study encompassed the performance characterization across 412 bacterial, viral, and parasitic species of interest via in silico simulations, demonstrating inclusivity and exclusivity performance.
Clinical Samples		Prior studies in human demonstrated the performance of mcfDNA across a range of conditions, including pneumonia, fever of unknown origin, invasive fungal disease, and sepsis.

Results



Matrix Equivalency. Comparison of mcfDNA quantification between human and porcine plasma showed a near-perfect linear relationship, with a regression slope of 1.003 and intercept of 4.1 MPM (95% CI for slope: [0.9895, 1.0158]; intercept: [-3.0, 11.1]). These results support equivalence between matrices, with no systematic bias detected.

95.4% Inclusivity

99.8% Exclusivity

98.9% PPV

17.7% CV

In silico inclusivity testing evaluated 195 representative taxa to confirm that laboratory-established limit-of-detection values remained robust across strain divergence and environmental background variation, detecting the correct target in 186 of 195 simulations with a positive predictive value of 98.94%.

In Silico exclusivity was evaluated by simulating high concentrations of mcfDNA from 412 off-panel taxa and assessing false positive rates under blinded analysis. The assay achieved a specificity of 99.76%, exceeding the predefined threshold of 92%.

Experimental precision was assessed across 20 batches, multiple operators, and two instruments using P13. Repeatability showed an average coefficient of variation (CV) of 17.7% and intermediate precision an average CV of 21.5%, with all microbes meeting the acceptance criterion of CV < 50%.