

Performance of a Novel Plasma-Based Next-Generation Sequencing Assay in Patients with Bacteremia

David K. Hong¹, Mickey Kertesz¹, Tim Blauwkamp¹, Cynthia Truong², and Niaz Banaei²

¹Karius, Inc., Menlo Park, CA, ²Stanford University Medical School, Stanford, CA

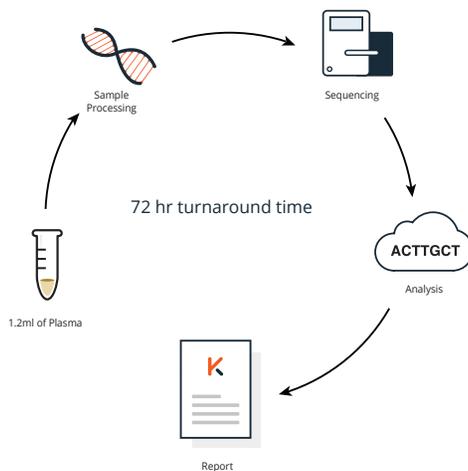
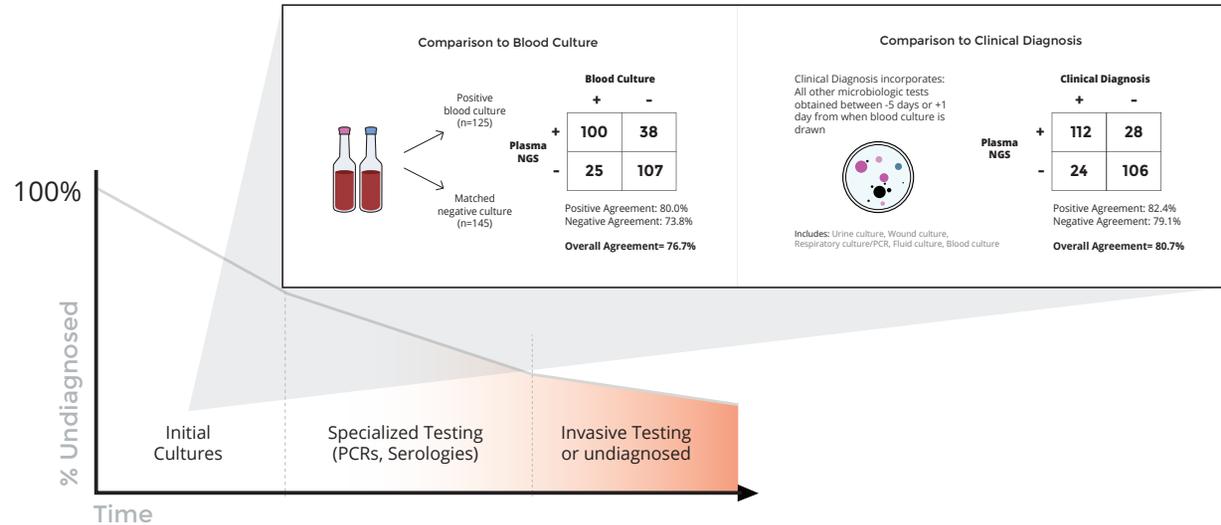
ABSTRACT

Background: Blood cultures are routinely obtained during the diagnostic evaluation for a number of infectious conditions such as sepsis, febrile neutropenia, and pneumonia. Despite its ubiquitous use, blood cultures have poor sensitivity due to a number of factors including previous antibiotic therapy or the presence of poorly-growing fastidious organisms. There is a need for more comprehensive diagnostic tests that can overcome these limitations to aid in guiding therapy.

Methods: We developed a plasma next-generation sequencing (NGS) assay capable of detecting over 5,000 bacteria, viruses and eukaryotic pathogens. To evaluate this assay in bacteremia, patients with multi-set positive and negative blood cultures were identified. We used our assay to analyze residual plasma samples that were obtained on the same day as the blood cultures. DNA was extracted from plasma and NGS performed. After filtering human reads, remaining reads were aligned to a pathogen sequence database. Relative abundance of each individual microorganism was calculated and pathogens estimated to be present with high statistical significance were identified.

Results: In comparing this novel NGS assay to blood culture and all other microbiologic data obtained from the patient, Positive Agreement was 82.4% (112/136) and Negative Agreement was 79.1% (106/134) with an Overall Agreement of 80.7%. When comparing directly to blood culture, there was agreement in culture-positive specimens in 100 of 125 specimens (80.0%). For blood culture-negative specimens, NGS did not detect a pathogen in 107 of 145 specimens (73.8%). Of the 38 false positives detected by the NGS assay as compared with blood culture, 10 were found to be true positives when compared with microbiologic data from the patient. These included detection of pathogens by plasma NGS that were found in endotracheal cultures, nasopharyngeal PCR, peritoneal cultures, or recently positive blood cultures.

Conclusion: We present data that show high concordance between a novel NGS assay and blood culture in patients with and without bacteremia. In addition, the assay was able to identify pathogens in plasma corresponding to pathogens identified from culture of other body sites. These results highlight the increased sensitivity in culture-negative infections and the broad nature of this assay.



Plasma NGS detected a broad array of pathogens in samples concordant with blood culture

Organism	Number of true positives
<i>Escherichia coli</i>	36
<i>Staphylococcus aureus</i>	16
<i>Enterococcus faecium</i>	8
<i>Klebsiella</i> species (<i>K. pneumoniae</i> or <i>K. oxytoca</i>)	6
<i>Pseudomonas aeruginosa</i>	6
<i>Streptococcus mitis</i> group	5
<i>Enterobacter</i> species (<i>E. cloacae</i> or <i>E. aerogenes</i>)	4
<i>Enterococcus faecalis</i>	3
<i>Streptococcus pneumoniae</i>	3
<i>Streptococcus dysgalactiae</i>	2
<i>Bacillus cereus</i>	2
Coagulase-negative <i>Staphylococcus</i> (<i>S. epidermidis</i>)	2
Other: <i>Lactobacillus paracasei</i> , <i>Citrobacter freundii</i> , <i>Morganella morganii</i> , <i>Streptococcus pyogenes</i> , <i>Salmonella enterica</i> , <i>Serratia marcescens</i> , <i>Rathia muclaginisosa</i>	7

Plasma NGS detected pathogens from deep infection sites in subjects with negative blood cultures

Blood Culture	Karius Test	Confirmatory Test	Day relative to blood culture
Negative	<i>Enterobacter</i> spp.	Sputum Culture	-5
Negative	<i>Enterobacter aerogenes</i>	Endotracheal culture	-2
Negative	Adenovirus	NP swab - respiratory/PCR panel	-1
Negative	<i>Enterobacter aerogenes</i>	Endotracheal culture	0
Negative	<i>Escherichia coli</i>	Endotracheal culture	0
Negative	<i>Moraxella catarrhalis</i>	Respiratory culture	-4
Negative	<i>F. nucleatum</i> , <i>B. thetaiotaomicron</i> , <i>B. fragilis</i> , <i>S. constellatus</i>	Pancreatic cyst culture - <i>B. fragilis</i>	-5
Negative		Abdominal fluid culture - <i>Streptococcus anginosus</i> , <i>Enterococcus faecalis</i>	-1
Negative	<i>S. intermedii</i>	Body fluid culture	+1
Negative	<i>Escherichia coli</i>	Blood Culture	-2
Negative	<i>Pseudomonas aeruginosa</i>	Urine Culture	0
Negative	<i>Escherichia coli</i>	Respiratory Culture	0
Coag-neg Staph	<i>Serratia marcescens</i>	Respiratory culture	0

SUMMARY

- Plasma NGS can detect a broad array of pathogens
- There is high concordance between a novel NGS plasma assay and blood culture in patients with and without bacteremia
- 26.3% of plasma NGS-positive/blood culture-negative samples were confirmed using additional microbiologic data
- Plasma NGS can detect pathogen DNA from deep body sites in patients with negative blood cultures