

Direct Detection and Quantification of Bacterial Cell-free DNA in Patients with Bloodstream Infection (BSI) Using the Karius Plasma Next Generation Sequencing (NGS) Test



Lisa Wanda, BS, BA¹, Felicia Ruffin, MSN, RN¹, Jonathan Hill-Rorie, BA¹, Pratik Shah, MS¹, Desiree Hollemon, MSN, MPH², Hon Seng, BS², David Hong, MD², Tim Blauwkamp, PhD², Mickey Kertesz, PhD² and Vance Fowler Jr., MD, MHS¹,



¹ Division of Infectious Diseases, Duke University Medical Center, Durham, NC, ² Karius, Inc., Redwood City, CA

Abstract

Background: Blood cultures can have low sensitivity if a patient is pre-treated with antibiotics. A molecular diagnostic for bloodstream infection (BSI) that can also quantitate pathogen DNA could be a useful tool in detecting and monitoring culture-negative infections.

Methods: We prospectively enrolled 108 patients (73 with culture confirmed BSI due to *Staphylococcus aureus* [n=36] or Gram negative bacilli [n=37] at baseline, and 35 with negative blood cultures) to evaluate the Karius plasma next generation sequencing (NGS) test to detect BSI. Blood samples from patients with confirmed BSI were collected for the study within one day of positive blood culture and then every 2-3 days. Cell-free DNA (cfDNA) was extracted from plasma and underwent NGS in the Karius CLIA/CAP laboratory (Redwood City, CA). After removal of human sequences, remaining reads were aligned against a curated pathogen database. Organisms present at a significance-level above a predefined threshold were reported. Quantity of cfDNA for each reported pathogen was expressed as molecules per microliter (MPM).

Results: When compared to baseline blood culture, the Karius plasma NGS test had a positive agreement of 74% (54/73) and negative agreement of 77% (27/35), **Table 2**. Pathogen cfDNA kinetics correlated with clinical findings, **Figure 1**. Overall, serially collected samples were positive by Karius plasma NGS testing significantly longer than blood culture (mean 6.5 days vs. 3.4 days, respectively; $p < 0.0001$), **Figure 2**. Patients with BSI were positive longer by Karius plasma NGS testing than blood culture for both *S. aureus* (mean 6.6 days vs. 3.4 days, respectively; $p < 0.001$) and gram-negative bacilli (mean 5.4 days vs. 1.6 days, respectively; $p < 0.001$). Pathogen cfDNA in BSI patients, quantified as MPM, declined over time during treatment. *S. aureus* MPM declined more slowly than gram-negative MPM at day 6 ($p < 0.001$), **Figure 3**.

Conclusions: The Karius plasma NGS test can directly detect pathogens in patients with BSI. Pathogen cfDNA signal in plasma remains positive longer than blood culture and, combined with quantification of pathogen cfDNA, could be a useful biomarker to aid in diagnosis and monitoring of infections, particularly in those with sterile blood cultures.

Patient Characteristics

ALL CULTURES (N=108)	
NEGATIVE BLOOD CULTURE (Control)	35 (32%)
GRAM NEGATIVE	37 (34%)
<i>Acinetobacter baumannii</i>	1 (1%)
<i>Burkholderia species</i>	1 (1%)
<i>Citrobacter amalonaticus</i>	1 (1%)
<i>Enterobacter aerogenes</i>	2 (2%)
<i>Enterobacter asburiae/cloacae</i>	3 (3%)
<i>Enterobacter gergoviae</i>	1 (1%)
<i>Escherichia coli</i>	11 (10%)
<i>Klebsiella pneumoniae</i>	5 (5%)
<i>Morganella morganii</i>	1 (1%)
<i>Prevotella melaninogenica</i>	1 (1%)
<i>Proteus mirabilis</i>	1 (1%)
<i>Providencia stuartii</i>	1 (1%)
<i>Pseudomonas aeruginosa</i>	5 (5%)
<i>Serratia marcescens</i>	2 (2%)
<i>Stenotrophomonas (xanthomonas) maltophil</i>	1 (1%)
STAPHYLOCOCCUS AUREUS	36 (34%)
MRSA	19 (18%)
MSSA	17 (16%)

Table 1: Blood culture types and associated organisms for all patients

Results

N=108	Blood Culture POSITIVE	Blood Culture NEGATIVE	
Karius POSITIVE	54	8	Pos Agreement: 74% Neg Agreement: 77%
Karius NEGATIVE	19	27	

Table 2: Blood Culture versus Karius Plasma NGS Test (Initial timepoint)

N=108	Blood Culture POSITIVE	Blood Culture NEGATIVE	
Karius POSITIVE	60	8	Pos Agreement: 82% Neg Agreement: 77%
Karius NEGATIVE	13	27	

Table 3: Blood Culture versus Karius Plasma NGS Test (All timepoints)

Results

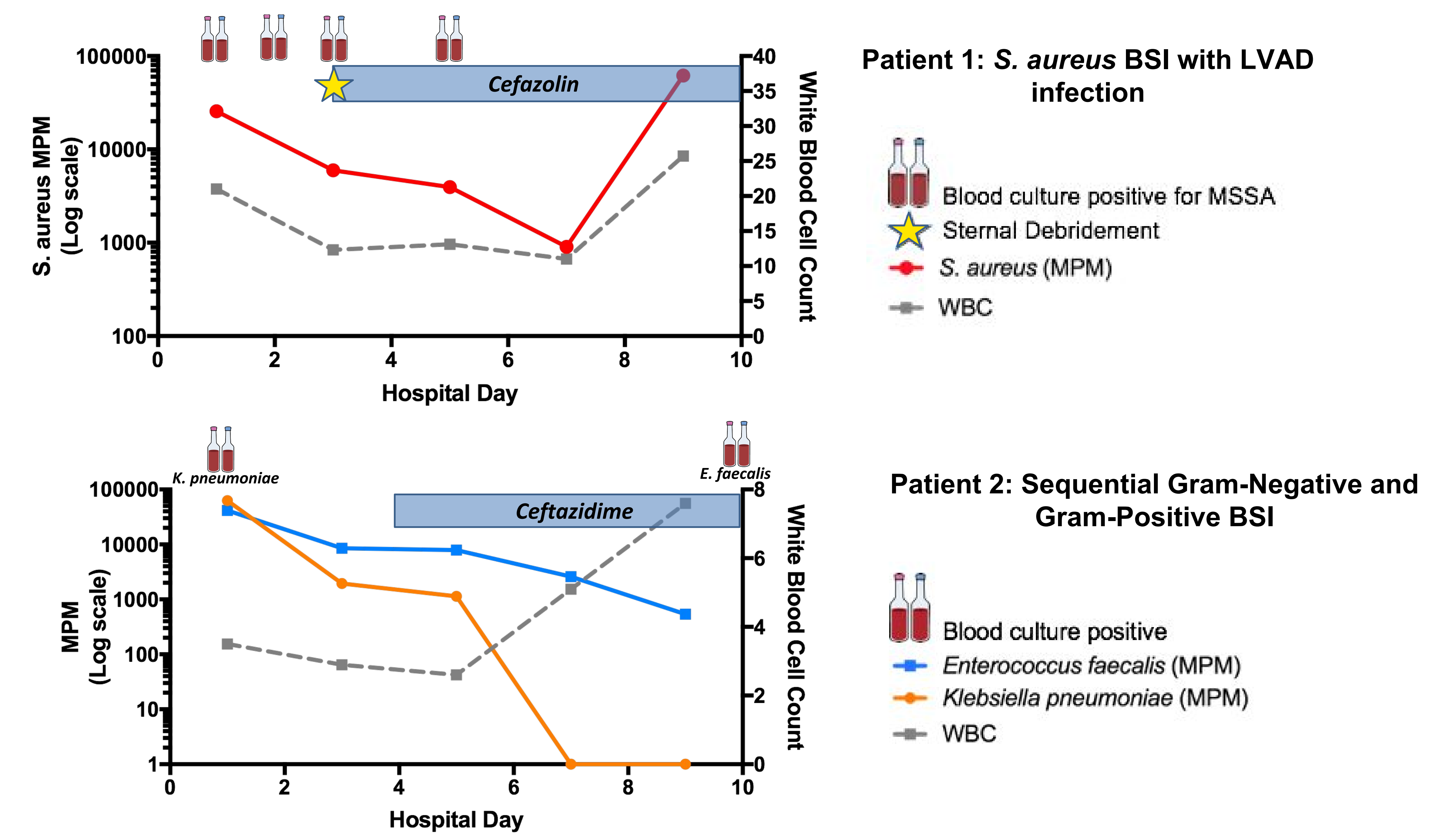


Figure 1: Kinetics of Pathogen Signal by Karius Plasma NGS Correlated with Clinical Findings

Plasma NGS Stays Positive Longer Than Blood Culture in Bloodstream Infections

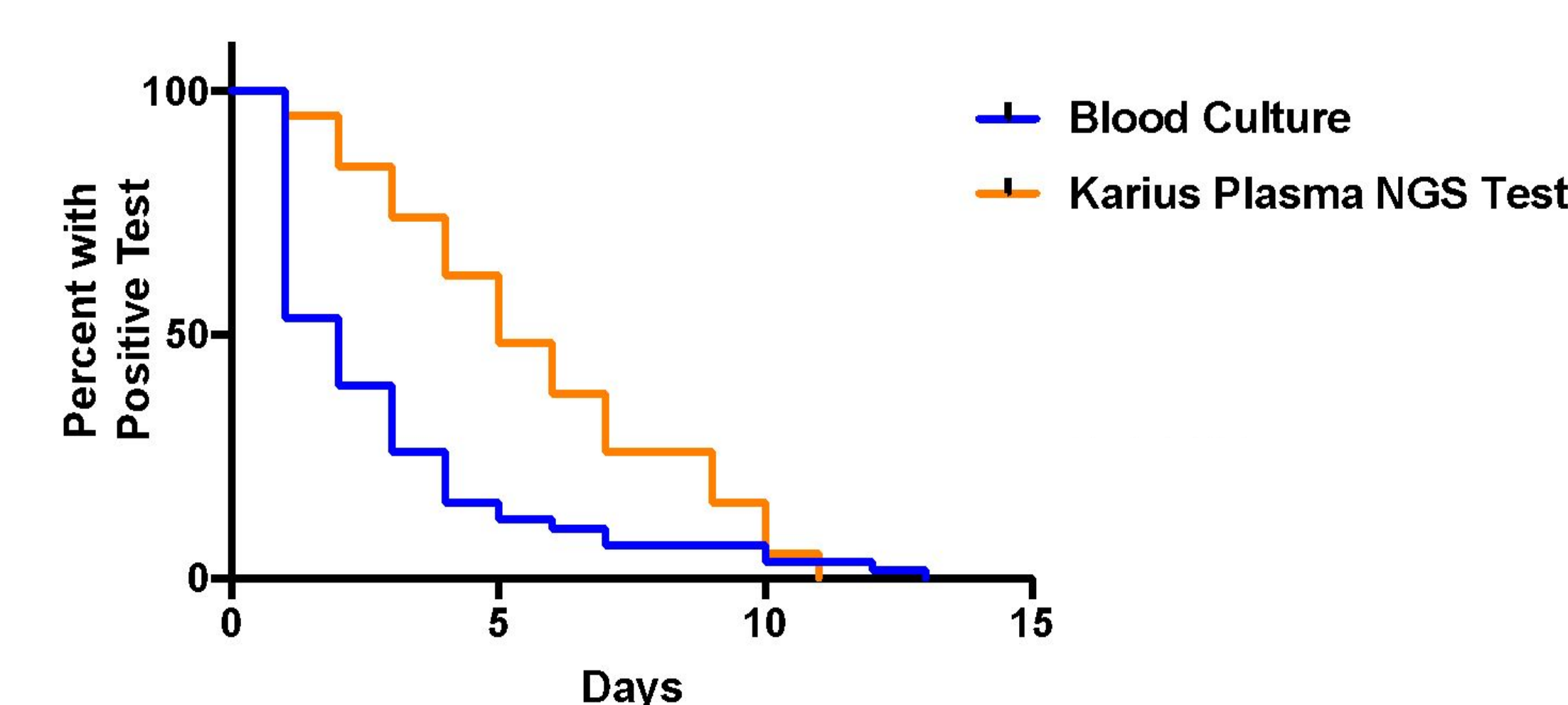


Figure 2: Duration of Positive Test by Method

Plasma NGS Stays Positive Longer Than Blood Culture for Both S. aureus and Gram-Negative Bloodstream Infections

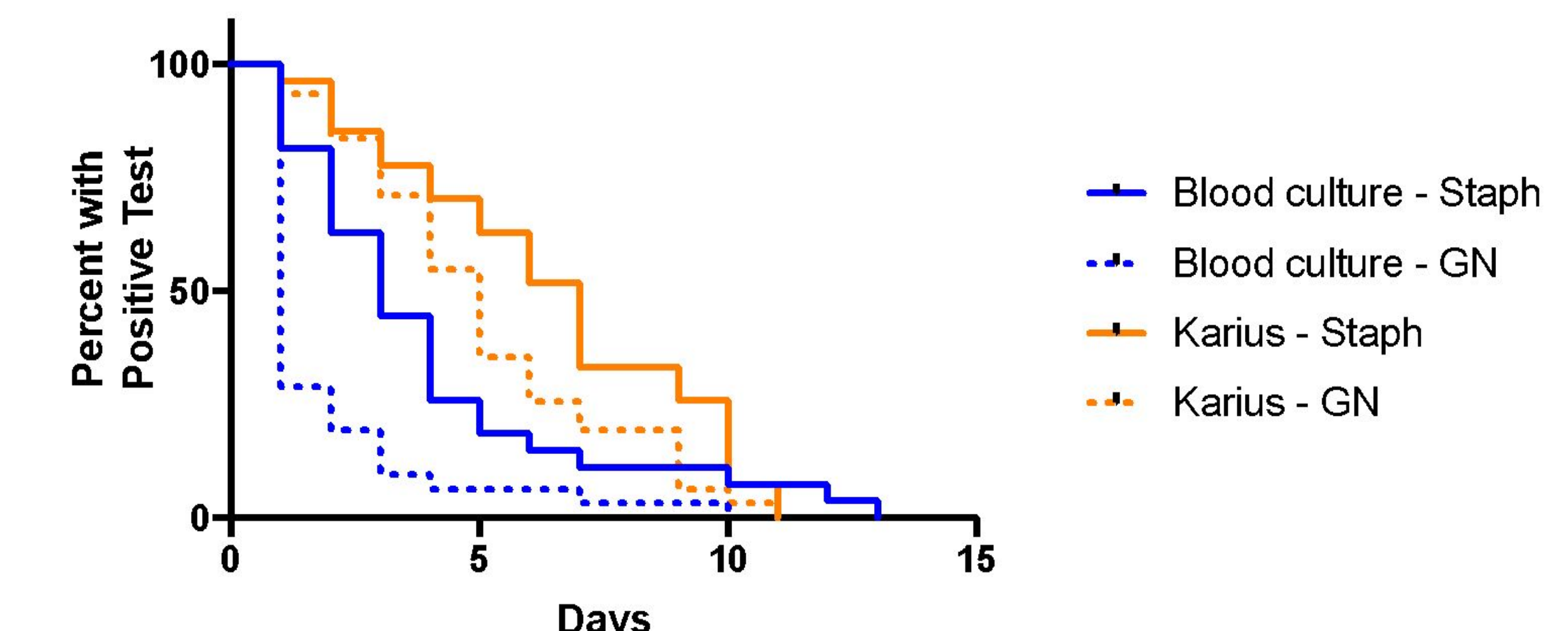
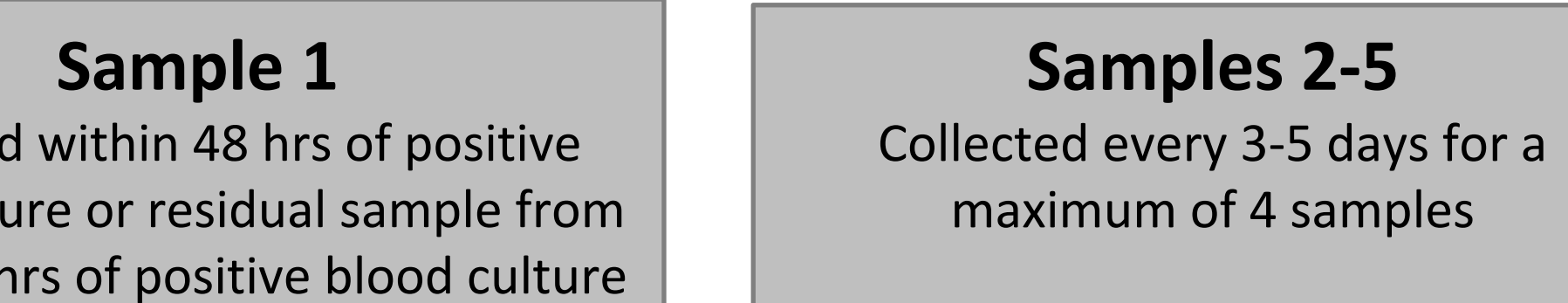


Figure 3: Duration of Positive Test by Method and Organism Type

Background and Methods

Study Design



Sample Processing and Workflow



Summary

- Karius plasma NGS test can directly detect pathogens in patients with bloodstream infections
- Pathogen cfDNA levels are dynamic during the course of infection
- In bloodstream infections, Karius plasma NGS signal stays positive 3.1 days longer than blood culture
- Kinetic analysis of pathogen cfDNA may provide useful clinical insights in patients with bloodstream infections