Identification and Antibiotic Resistance Profiling of Bacterial Pathogens Directly from Whole Human Blood Utilizing Blood2Bac

Abstract #5381

Matthew E. Turner[1], Paul Knysh[1], Jason Wittenbach[1], Defne Surujon[1], Ian Herriott[1], Emily MacLeod[1], Cabell Maddux[1], Rachel Martin[1], Cristina Rushton[1], Danielle Peterson[1], Mohamad Sater[1], Linnea Sahlberg[1], Alfred Wong[1], Kaitlin Woodruff[1], Claire Zimmerman[1], Nicole Billings[1], Miriam H. Huntley[1], Manoj Nair[1], Douglas S. Kwon[1,2]

[1]*Day Zero Diagnostics- Boston, Massachusetts,* [2]*Ragon Institute of MGH, MIT, and Harvard- Cambridge, Massachusetts*

Abstract

Bloodstream infections (BSI) are a leading cause of patient mortality with early diagnosis and pathogen identification key to improving clinical outcomes. However, longer turn-around times associated with culture-based diagnostics limit the ability of clinical labs to provide timely information to clinicians. Moreover, delayed treatment for patients significantly impacts clinical outcomes with septic shock mortality rates increasing by 8% per hour without appropriate antibiotics. Day Zero Diagnostics has developed Blood2Bac™, a culture-free, pathogen-agnostic technology which enriches bacterial pathogens directly from whole human blood and utilizes bacterial whole genome sequencing and proprietary algorithms to provide sensitive identification of pathogens and antimicrobial resistance (AMR) information at single digit CFU/mL levels. Performance assessment of the Blood2Bac™ process made across 26 priority BSI species demonstrated analytical sensitivity at or below 5 CFU/mL concentration with average genomic coverage of 99.14% for 23 out of 26 of the tested strains. The ability to discriminate between individual species was demonstrated within 5 sets of polymicrobial samples, each containing two different priority BSI species. Additionally, assessment of Blood2Bac™ and the Keynome® ID algorithm for species identification was also made on a blinded panel of 40 distinct strains provided through the IHMA biobank at 97.5% accuracy (positive ID for 39/40 blinded organisms). Further, the ability of the Keynome® gAST algorithm to determine antibiotic susceptibility profiles of pathogens directly from blood tested on 50 bacterial strains spiked into whole blood at 5.5-21.5 CFU/mL levels demonstrated Categorical Agreement of 91.3% with phenotypic AST. As a whole, these results demonstrate the sensitivity and accuracy of Blood2Bac™ for the identification and AMR profiling of BSI pathogens in the single digit bacterial CFU/mL range direct from whole human blood.

Methods

Blood2Bac™ (B2B) analytical performance was assessed in monomicrobial samples, across 26 bacterial pathogens, and polymicrobial samples, across 5 high priority BSI species, by creating contrived spike-ins containing concentrations between 1-10 CFU/mL of bacteria. Contrived samples were then processed through WGS utilizing Oxford Nanopore Technologies (ONT) and analyzed using the DZD bioinformatic species ID algorithm Keynome® ID.
Assessment of Blood2Bac™ against a blinded set of bacterial pathogens was then assessed in a similar manner using a panel of unknown pathogens provided to Day Zero Diagnostics from the Combatting Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) via IHMA. Blinded species were then spiked into contrived samples at concentrations between 6-12 CFU/mL. Contrived samples were then processed as indicated above.

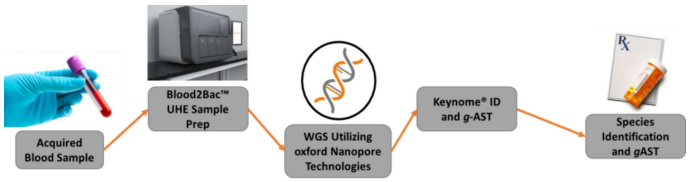

Figure 1: Schematic for Blood2Bac™ Sample Processing and Analysis.

#	Strain Species	Replicates Tested	Analytical Sensitivity (CFU/mL)	Tested Range (CFU/mL)	Avg. 1X Genome Coverage
1	*Acinetobacter baumannii*	9	≤2	0.96-9.81	93.20%
2	*Bacillus cereus*	3	≤2	1.3-14.1	99.93%
3	*Citrobacter freundii*	4	≤2	0.75-1.55	98.52%
4	*Clostridium perfringens*	4	≤2	1.03-1.08	96.47%
5	*Enterobacter hormaechei*	9	≤2	1.35-9	99.71%
6	*Enterococcus faecalis*	8	≤2	1.08-8.27	99.98%
7	*Enterococcus faecium*	9	≤2	0.91-12.4	98.18%
8	*Escherichia coli*	12	≤2	1.97-12.36	99.96%
9	*Haemophilus influenzae*	9	≤2	1.06-8.86	99.19%
10	*Klebsiella aerogenes*	4	≤2	1.26-1.7	99.96%
11	*Klebsiella pneumoniae*	9	≤2	1.11-13.7	98.95%
12	*Morganella morganii*	12	≤2	0.74-10.59	99.77%
13	*Proteus mirabilis*	10	≤2	1.95-23.39	98.08%
14	*Pseudomonas aeruginosa*	9	3	0.88-7.04	26.17%
15	*Pseudomonas putida*	4		0.88-1.1	22.87%
16	*Serratia marcescens*	9	≤2	1.17-9.62	96.84%
17	*Staphylococcus aureus*	9	≤2	1.33-10.39	99.66%
18	*Staphylococcus epidermidis*	9	≤2	1.09-9.36	97.29%
19	*Staphylococcus lugdunensis*	9	≤2	1.06-10.59	99.98%
20	*Staphylococcus simulans*	9	≤2	1.13-9.06	99.98%
21	*Stenotrophomonas maltophilia*	9	5	1.43-8.59	12.95%
22	*Streptococcus agalactiae*	9	≤2	0.95-16.11	99.98%
23	*Streptococcus anginosus*	9	≤2	0.95-9.81	97.77%
24	*Streptococcus mutans*	9	≤2	1.19-10.07	97.45%
25	*Streptococcus pneumoniae*	3	≤2	1	99.98%
26	*Streptococcus pyogenes*	9	≤2	1.08-10.12	99.90%

Table 1: Blood2Bac™ analytical performance across 26 bacterial BSI pathogens. At least 3 donor replicates were tested for each species using a contrived spike-in model. Measured analytical sensitivity depicts the minimal CFU/mL concentration at which each species is reliably detected within the indicated number of tests. Indicated genomic coverage represents the proportion of the genome covered at least once by sequenced reads following test completion.

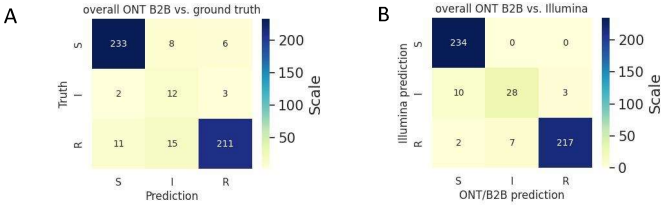

Figure 2: Keynome® *g*-AST Model Performance against Blood2Bac/ONT and Illumina Processed Samples. **(A)** Contrived whole blood spike-ins processed through Blood2Bac™ at bacterial concentrations between 5-20 CFU/mL and sequenced using ONT or **(B)** purified bacterial gDNA sequenced using Illumina were analyzed through DZD's Keynome® *g*-AST model.

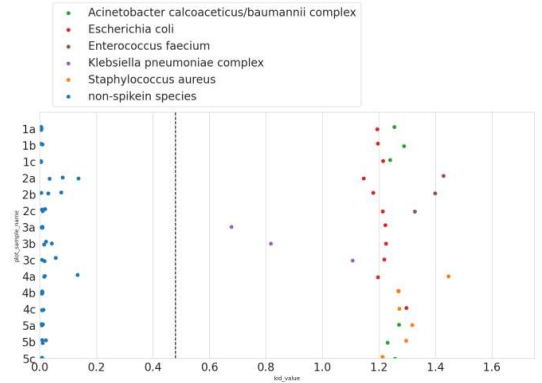

Figure 3: Blood2Bac™ Performance In Polymicrobial Samples. Contrived blood samples containing a combination of 2 priority BSI species between 1-13.5 CFU/mL were spiked into whole human blood and processed through Blood2Bac™. Demonstrated results indicate 100% sensitivity for ID of each unique pathogen introduced into the sample, with clear separation of noise against other, non-target organisms.

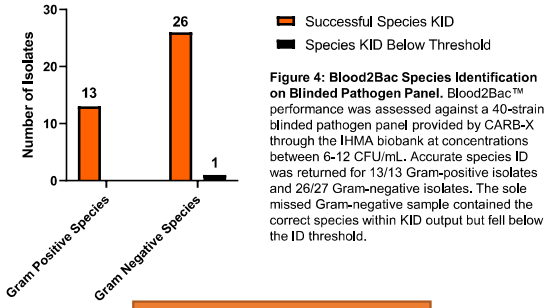

Figure 4: Blood2Bac Species Identification on Blinded Pathogen Panel. Blood2Bac™ performance was assessed against a 40-strain blinded pathogen panel provided by CARB-X through the IHMA biobank at concentrations between 6-12 CFU/mL. Accurate species ID was returned for 13/13 Gram-positive isolates and 26/27 Gram-negative isolates. The sole missed Gram-negative sample contained the correct species within KID output but fell below the ID threshold.

Conclusion

These results demonstrate the ability of the Blood2Bac™ assay to accurately identify a wide range of bacterial pathogens at single digit CFU/mL concentrations, correctly identify polymicrobial mixtures of multiple BSI pathogens, and generate accurate AST predictions via Keynome® *g*-AST at single digit CFU/mL inputs. The ability of Bldd2Bac to recover whole genomes of pathogens in a rapid manner direct from blood makes it an exciting prospect for BSI diagnosis and treatment in a clinical setting.