#01161

Rapid species detection and antibiotic susceptibility profiling for bloodstream infections directly from whole blood at single digit pathogen concentrations

04. Diagnostic microbiology
04h. Clinical metagenomics

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Background

Sequencing-based diagnostics are an emerging method for clinical diagnosis that leverage an agnostic approach for the identification of pathogens. However, there are barriers to the realization of sequencing-based diagnostics, including separation of true signal of pathogens from background commensals and billion-fold excess host DNA. Blood2Bac™ is a rapid, culture-free, pathogen-agnostic technology that enriches bacterial and fungal DNA by up to 10 logs directly from whole blood. This ultra high enrichment enables rapid whole genome sequencing (WGS) and computational analysis with a machine learning-based software package called Keynome® to provide accurate species identification (ID) and antimicrobial susceptibility (AST) directly from whole blood at single digit CFU/mL pathogen concentrations in <8 hours.

Methods

A total of 35 BSI pathogens (29 bacteria and 6 yeasts), were spiked into healthy donor blood at concentrations ranging 0.44-5.63 CFU/mL. Samples were processed through Blood2Bac™ and whole genome sequenced on PromethION 24 using the LSK114 kit (Oxford Nanopore Technologies). Data was processed through Keynome® for ID and predictive AST.

Results

We demonstrate the ability to accurately identify 35 bacterial and fungal BSI pathogens at single digit CFU/mL concentrations (Table 1). The majority of samples resulted in near complete genome recovery, with an average 1x coverage of 94.3% for bacteria and 99.3% for fungi (Figure 1). We demonstrate a high specificity of 97.1% with just 3 false positive calls across 105 tested samples. For AST, 109 AST predictions on 47 individual bug/drug combinations across 10 BSI strains were compared to phenotypic results with 92.7% categorical agreement. Total turnaround time for Blood2Bac™ was an average of 5.77 hours (Figure 2) and we previously demonstrated a singleplex time of 2 hours for library preparation, ONT sequencing, and computational analysis.

Conclusions

We successfully identified both bacterial and fungal BSI pathogens at single digit concentrations with high accuracy. The ability of Blood2Bac™ to rapidly recover whole pathogen genomes and for Keynome® to provide accurate ID and AST predictions demonstrates their potential to have a high impact on the clinical management of BSIs utilizing whole genome sequencing.

Table 1

Strain Species	Tested Conc. (CFU/mL)	Avg. 1X Genome Coverage	Strain Species	Tested Conc. (CFU/mL)	Avg. 1X Genome Coverage
Acinetobacter baumannii	2.9	93.21%	*Listeria monocytogenes*	2.1	99.97%
Acinetobacter ursingii	2.0	99.03%	*Morganella morganii*	1.3	99.99%
Bacillus cereus	5.6	95.62%	*Proteus mirabilis*	2.6	99.94%
Candida albicans	1.9	99.83%	*Pseudomonas aeruginosa*	3.2	24.93%
Candida auris	2.6	99.27%	*Pseudomonas putida*	4.6	81.41%
Candida glabrata	2.2	99.49%	*Raoultella ornithinolytica*	3.0	91.92%
Candida krusei	2.2	99.38%	*Serratia marcescens*	2.8	99.89%
Candida parasilopsis	2.1	99.09%	*Staphylococcus epidermidis*	1.6	99.87%
Candida tropicalis	3.3	98.67%	*Staphylococcus lugdunensis*	3.7	99.99%
Citrobacter freundii	2.2	98.79%	*Staphylococcus aureus*	2.7	100.00%
Citrobacter koseri	0.4	97.37%	*Streptococcus dysgalactiae*	2.3	100.00%
Enterobacter cloacae	2.7	99.44%	*Streptococcus agalactiae*	2.1	99.86%
Enterococcus faecalis	2.4	99.98%	*Streptococcus anginosus*	2.4	97.59%
Enterococcus faecium	2.3	98.18%	*Streptococcus oralis*	1.8	97.43%
Escherichia coli	1.8	96.34%	*Streptococcus pyogenes*	4.5	100.00%
Klebsiella aerogenes	2.6	87.10%	*Streptococcus mutans*	1.6	99.81%
Klebsiella oxytoca	4.3	91.49%	*Streptococcus sanguinis*	2.8	94.89%
Klebsiella pneumoniae	1.6	83.39%			

Table 1: Blood2Bac™ experimental results from contrived blood samples. 35 strains representing 35 distinct species were spiked into whole blood at the indicated concentrations for 3 replicates of testing. Average genome recovery (1X alignment to strain reference assembly) is indicated in the last column, showing near complete recovery across most species.

Figure 1

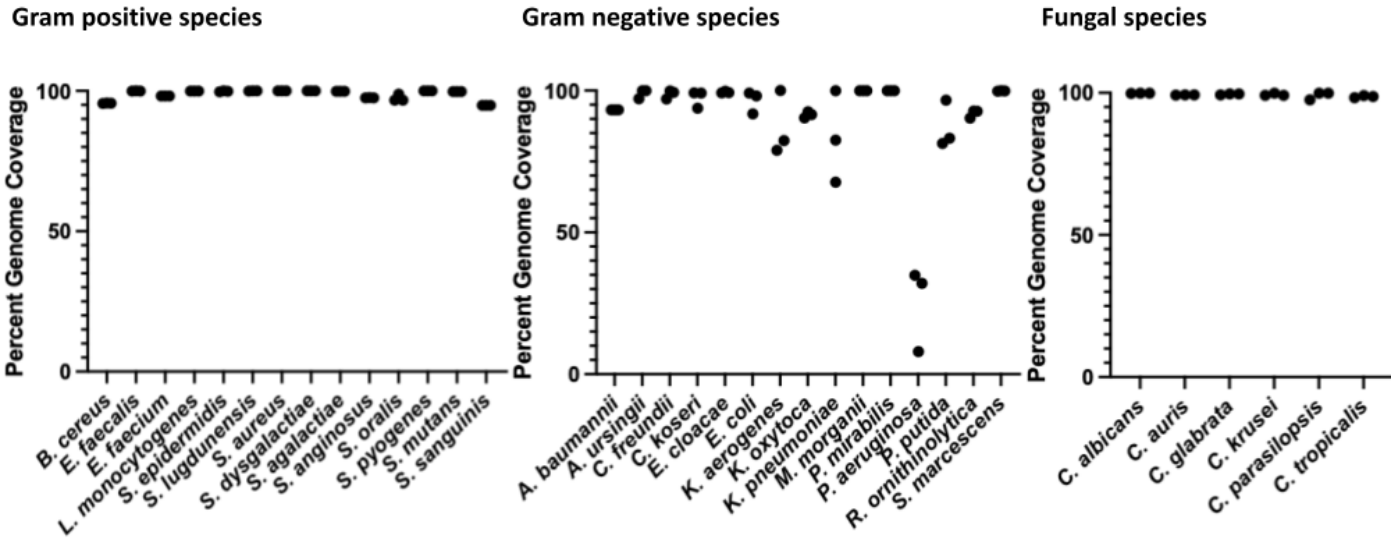

Figure 1: Blood2Bac™ experimental results from contrived blood samples. 35 strains representing 35 distinct species were spiked into whole blood at various concentrations <6 CFU/mL for 3 replicates of testing. Genomic recovery at 1x depth for Gram positive (left), Gram negative (middle), and fungal species was measured by aligning the sequence of each contrived sample back to the strain's de novo assembly genome (created previously using short-read Illumina sequencing).

Figure 2

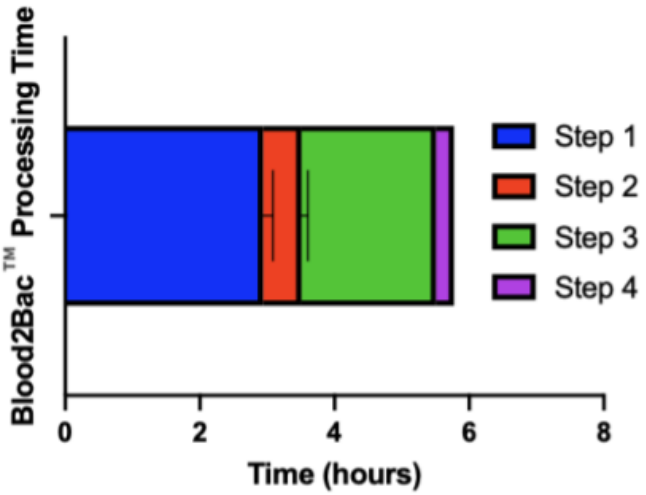

Figure 2: Processing time required for Blood2Bac™ assay. The time needed for the completion of the Blood2Bac™ process is broken down into the 4 major assay steps. Reported times represent the averages of N=66 individually processed samples. Samples were multiplexed for sequencing and processed in batch, so follow-up library preparation, sequencing, and computational analysis times were not optimized for turnaround time in this experiment; in prior work we demonstrated a 2 hour window per sample for these processes.