Rapid ultra-high enrichment of bacterial pathogens at low concentration from whole blood for species ID and AMR prediction using Oxford Nanopore sequencing

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Introduction

There is an urgent need for rapid and accurate diagnostics to guide targeted therapy for blood stream infections (BSIs). BSIs are among the leading causes of morbidity and mortality, yet the status quo culture-based diagnostics have a multi-day turnaround time (TAT) and limited ability to guide antibiotic therapy. Day Zero Diagnostics has developed Blood2Bac™, a species-agnostic culture-free method to enrich bacterial DNA directly from whole blood samples by a factor of 100,000,000. When Blood2Bac is coupled with ONT rapid whole genome sequencing and Day Zero Diagnostics' algorithmic tools, we can determine bacterial species identification (ID) and antimicrobial resistance (AMR) within 10 hours of blood draw, a drastic decrease in diagnostic TAT. Here we demonstrate the performance of our technology on both contrived (spike-in) samples and clinical samples.

Low CFU Validation on Contrived Blood Samples

We first evaluated the performance of Blood2Bac + MinION sequencing and custom algorithmic tools on a set of contrived samples, prepared with 26 clinically relevant bacterial pathogens (both gram negative and gram positive) spiked into whole blood at very low concentrations. We report ultra high bacterial DNA enrichment and pathogen whole genome coverage at bacterial concentrations 1-4 CFU/mL (**Table 1**). In 89 of 96 samples there were more sequenced bases originating from the spiked pathogen of interest (POI) than bases originating from the human genome, and the average ratio of POI bases to human bases was greater than 28. Aligning the sequenced reads to the previously assembled genomes of the spiked pathogens, we found that on average **99% of the genome was covered** at 1x depth and 94% at 5x depth. For five of the species tested, we ran the sequencing output of samples through Keynome® AMR, our custom machine learning tool for analyzing nanopore data to determine the AMR profile. We found the **AMR predictions had 100% accuracy** for all 22 drug/species AMR combinations with both available phenotypes and Keynome predictions (**Table 2**).

Table 1: Whole Genome Recovery from Whole Blood with Low CFU Spike-ins

		Average Across *N* Samples			
Species	***N***	**Spike-in CFU/mL**	**POI Bases / Human Bases**	**1x Genome Coverage (%)**	**5x Genome Coverage (%)**
Acinetobacter baumannii	5	3.12	33.19	99.96	99.52
Citrobacter freundii	2	1.00	44.24	99.16	96.67
Citrobacter koseri	2	1.80	12.56	99.88	97.66
Enterobacter cloacae	11	1.98	7.73	95.25	82.95
Enterococcus faecalis	2	1.90	122.08	99.85	99.64
Enterococcus faecium	2	1.06	20.04	99.98	99.27
Enterococcus gallinarum	2	0.83	2.44	99.79	99.38
Escherichia coli	6	2.08	105.52	99.90	98.40
Klebsiella aerogenes	2	1.30	5.52	99.80	98.23
Klebsiella oxytoca	2	1.00	12.35	98.45	85.40
Klebsiella pneumoniae	8	1.81	12.20	97.94	84.61
Morganella morganii	2	0.94	48.13	99.79	96.82
Pasteurella multocida	2	1.02	20.66	99.83	99.82
Raoultella ornithinolytica	2	1.30	25.82	99.59	97.05
Staphylococcus aureus	4	3.77	5.99	99.48	96.88
Staphylococcus epidermidis	6	2.46	14.34	98.21	91.56
Staphylococcus haemolyticus	2	0.69	13.49	99.96	99.90
Staphylococcus saprophyticus	2	1.13	19.64	98.85	94.72
Staphylococcus warneri	2	1.20	23.57	99.97	99.93
Streptococcus agalactiae	6	1.82	81.61	98.71	98.68
Streptococcus anginosus	2	1.13	2.62	99.94	99.86
Streptococcus intermedius	2	1.02	17.21	99.93	99.91
Streptococcus oralis	6	1.37	25.52	98.54	98.10
Streptococcus parasanguinis	2	1.27	7.89	99.61	99.15
Streptococcus pneumoniae	6	1.49	6.53	99.25	95.02
Streptococcus pyogenes	2	1.29	5.14	99.84	98.90

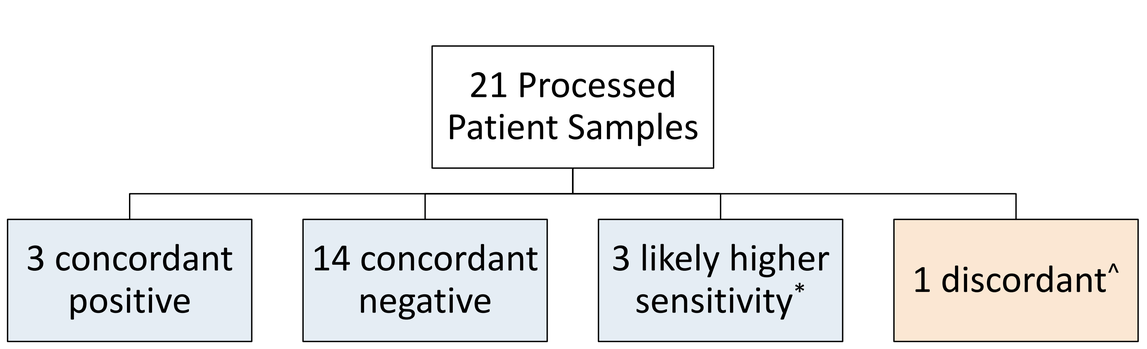
Table 2: AMR Prediction Accuracy with Keynome

	aztreonam	cefoxitin screen	ciprofloxacin	ceftriaxone	clindamycin	erythromycin	gentamicin	levofloxacin	methicillin	moxifloxacin	tetracycline	TMP/SMX	vancomycin	CFU/mL
E. coli			100%					100%						2 (*N*=6)
K. pneumoniae	100%			100%										2 (*N*=8)
S. aureus		100%	100%		100%	100%	100%	100%	100%	100%	100%	100%		4 (*N*=4)
S. epidermidis		100%			100%	100%			100%					2 (*N*=6)
S. agalactiae						100%								2 (*N*=6)
E. faecium			100%					100%					100%	1 (*N*=2)

Clinical Study Demonstrates High Species ID Concordance

We also evaluated the Blood2Bac platform in a clinical study at a Boston Medical Center. To date, we have tested whole blood specimens from 21 hospitalized adult patients with suspected and/or documented BSI. Specimens were processed with Blood2Bac + MinION sequencing. When we compared our results to the most recent blood cultures drawn within 24 hours of specimen collection (**Figure 1**), Keynome ID called concordant species ID in 17/21 specimens: 3 were concordant positive ID of matching species, and 14 were concordant negative ID (no BSI). In 3/21 discordant samples, Keynome ID called a pathogen present while same-day blood cultures were negative. However, the Keynome IDs for all 3 samples matched earlier positive blood culture ID results from 21-28 hours prior to specimen collection, suggesting that **Blood2Bac may have higher sensitivity than blood culture.** In 1/21 specimens, Keynome ID called no pathogen present while blood culture from eight hours prior had a positive result. Notably, a blood culture 19 hours after specimen collection was negative, and the patient had been on empiric therapy for roughly 8 days prior to specimen collection.

Figure 1: Clinical Study Species ID Concordance with Blood Culture ID



* Positive Keynome ID disagreed with most recent prior blood culture (which was negative) but was concordant with earlier positive blood culture ID 21-28 hours prior

^ Negative Keynome ID disagreed with same day blood culture (which was positive) but agreed with blood culture 19 hours after collection

Conclusion

Blood2Bac, in combination with ONT sequencing and Keynome analysis, shows high potential for filling the urgent need for a rapid and accurate diagnostic for blood stream infections.

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