Accurate species identification and antibiotic susceptibility prediction for multiple pathogens in contrived polymicrobial blood samples using whole genome Oxford Nanopore sequencing

Danielle Peterson*, Defne Surujon*, Connor Capitolo*, Claire Zimmerman*, Emily MacLeod*, Emily Condon*, Maria Ramos*, Allison Brookhart*, Talia Hollowell*, Alfred Wong*, Ian Herriott*, Nicole Billings*, Mohamad Sater*, Cabell Maddux*, Jason Wittenbach*, Miriam Huntley*
*Day Zero Diagnostics, Inc.

Introduction

- Polymicrobial infections – defined broadly as infection by multiple microorganisms – are associated with increased morbidity, length of hospital stay and duration of intensive care. These infections are typically challenging to diagnose with standard blood culture systems. In addition, because microbes can share similar resistance markers, molecular methods can struggle to attribute resistance to the correct species when multiple organisms are present.
- Day Zero Diagnostics® is developing a diagnostic for identifying bacteria in bloodstream infections using ultra-high pathogen enrichment (Blood2Bac™) followed by whole genome sequencing of bacteria on the Oxford Nanopore Technologies (ONT) platform. Together with Keynome® ID, our species identification algorithm, our end-to-end process can detect the correct species in whole blood in hours, compared to blood culture, which can often take days. Keynome® *g*-AST (genomic Antibiotic Susceptibility Testing), our proprietary machine learning algorithm, predicts antibiotic resistance using whole genome sequencing data.

Methods

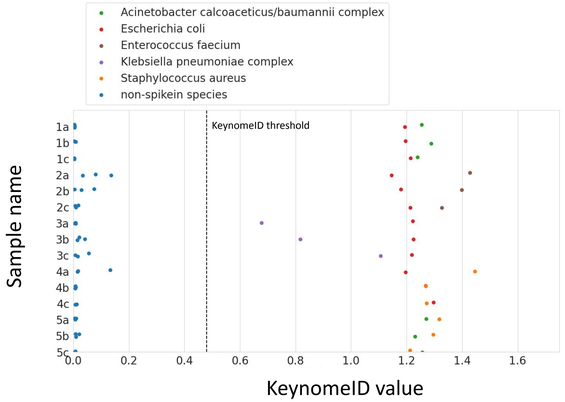
- We tested our entire end-to-end process (Blood2Bac™, Keynome® ID, and Keynome® *g*-AST) on a set of 15 contrived polymicrobial blood samples where 5 pathogen pairs were spiked in at clinically relevant bacterial loads (1-13 CFU/mL).

Sample name	Species	KeynomeID value	Spike-in concentration	1x coverage of closest matching reference genome	5x coverage of closest matching reference genome
1a	*Acinetobacter calcoaceticus/baumannii complex*	1.25	7.31 CFU/mL	0.97	0.97
1a	*Escherichia coli*	1.19	2.41 CFU/mL	1.00	1.00
1b	*Acinetobacter calcoaceticus/baumannii complex*	1.29	6.18 CFU/mL	0.97	0.97
1b	*Escherichia coli*	1.20	3.77 CFU/mL	1.00	1.00
1c	*Acinetobacter calcoaceticus/baumannii complex*	1.24	6.18 CFU/mL	0.97	0.97
1c	*Escherichia coli*	1.21	3.77 CFU/mL	1.00	1.00
2a	*Enterococcus faecium*	1.43	7.73 CFU/mL	0.91	0.90
2a	*Escherichia coli*	1.15	2.78 CFU/mL	1.00	1.00
2b	*Enterococcus faecium*	1.40	7.73 CFU/mL	0.91	0.90
2b	*Escherichia coli*	1.18	2.78 CFU/mL	1.00	1.00
2c	*Enterococcus faecium*	1.33	6.82 CFU/mL	0.91	0.90
2c	*Escherichia coli*	1.21	2.41 CFU/mL	1.00	1.00
3a**	*Escherichia coli*	1.22	3.77 CFU/mL	1.00	1.00
3a**	*Klebsiella pneumoniae complex*	0.68	1.19 CFU/mL	0.95	0.76
3b**	*Escherichia coli*	1.23	2.97 CFU/mL	1.00	1.00
3b**	*Klebsiella pneumoniae complex*	0.82	1.94 CFU/mL	0.98	0.85
3c**	*Escherichia coli*	1.22	2.97 CFU/mL	1.00	1.00
3c**	*Klebsiella pneumoniae complex*	1.11	1.94 CFU/mL	1.00	0.98
4a	*Staphylococcus aureus*	1.32	10.93 CFU/mL	1.00	1.00
4a	*Acinetobacter calcoaceticus/baumannii complex*	1.27	11.39 CFU/mL	0.97	0.97
4b	*Staphylococcus aureus*	1.30	10.93 CFU/mL	1.00	1.00
4b	*Acinetobacter calcoaceticus/baumannii complex*	1.23	11.39 CFU/mL	0.97	0.97
4c	*Acinetobacter calcoaceticus/baumannii complex*	1.26	9.58 CFU/mL	0.97	0.97
4c	*Staphylococcus aureus*	1.21	10.34 CFU/mL	1.00	1.00
5a	*Staphylococcus aureus*	1.45	8.8 CFU/mL	1.00	1.00
5a	*Escherichia coli*	1.20	2.6 CFU/mL	1.00	0.98
5b	*Staphylococcus aureus*	1.27	13.38 CFU/mL	1.00	1.00
5b	*Escherichia coli*	1.27	2.53 CFU/mL	1.00	1.00
5c	*Escherichia coli*	1.30	2.53 CFU/mL	1.00	1.00
5c	*Staphylococcus aureus*	1.27	13.38 CFU/mL	1.00	1.00

** All *Escherichia coli* and *Klebsiella pneumoniae* complex spike-in combinations (Samples 3a, 3b, 3c) were excluded from *g*AST analysis because both species are in the same family.

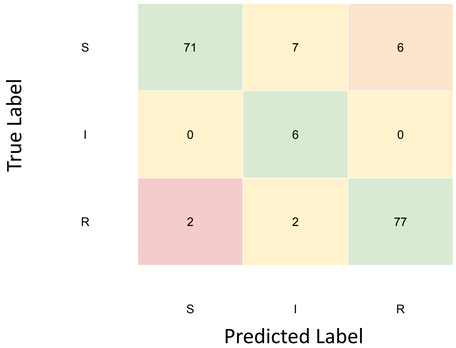
Keynome® ID results

- We were able to detect all organisms in contrived polymicrobial blood samples with 100% sensitivity and 100% specificity (see figure below, KID threshold=0.48, dashed line).
- We were able to retain >90% 1x coverage for all spike-in strains (see Table)



Keynome® *g*-AST results

- Keynome® *g*-AST achieves 90.06% categorical agreement in these challenging samples (samples that contain species from the same family were excluded from *g*-AST analysis).
- n = 12 samples, 4 polymicrobial combinations, 25 species-drug combinations, 147 predictions



Conclusions

We show that the Keynome® pipeline is able to robustly identify polymicrobial species directly from a blood sample, allowing accurate predictions of antibiotic resistance phenotypes for all tested species present in polymicrobial blood samples. These advancements in our diagnostic could enable clinicians to treat patients more quickly, reduce the use of unnecessary antibiotics and improve health-outcomes.

DAYZERO DIAGNOSTICS