

# Direct-from-Blood Microbial Sequencing Assay for Pathogen and Antibiotic Resistance Detection in Bloodstream Infections

Ian C. Herriott<sup>1</sup>, D. Kwon<sup>1,2</sup>, Manoj Nair<sup>1</sup>, Matthew Turner<sup>1</sup>, Defne Surujon<sup>1</sup>, Emily Macleod<sup>1</sup>, Rachel Martin<sup>1</sup>, Cristina Rushton<sup>1</sup>, Alfred Wong<sup>1</sup>, Allison Brookhart<sup>1</sup>, Talia Hollowell<sup>1</sup>, Kaitlin Woodruff<sup>1</sup>, Claire Zimmerman<sup>1</sup>, Linnea Sahlberg<sup>1</sup>, Cabbel Maddux<sup>1</sup>, Mohamad R. Sater<sup>1</sup>, Miriam H. Huntley<sup>1</sup>

<sup>1</sup>Day Zero Diagnostics - Boston (United States); <sup>2</sup>Ragon Institute of MGH, MIT and Harvard - Cambridge (United States)

## Introduction

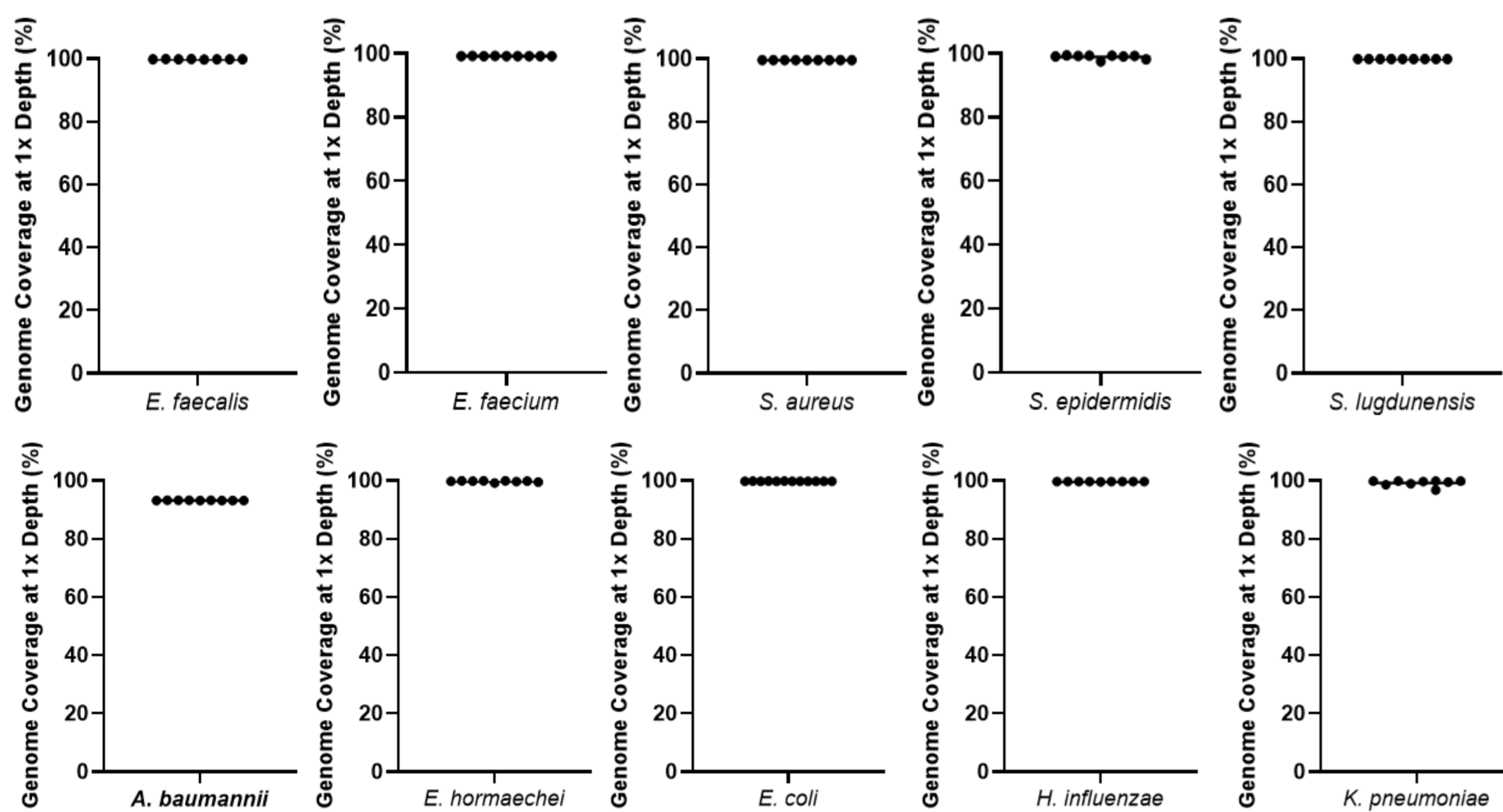
Bloodstream infections (BSIs) are a leading cause of morbidity and mortality that require rapid identification of the infecting pathogen to guide treatment. However, nearly all available diagnostics for BSIs rely on blood culture and so are inherently slow, requiring 1-2 days for bacterial growth. Achieving whole genome recovery of bacterial pathogens directly from blood is challenging due to (1) low *absolute abundance*, with clinically relevant loads of 1 CFU/mL and only 10s of femtograms of bacterial DNA; (2) low *relative abundance*, as human DNA outnumbers DNA in clinical blood samples by 8-9 orders of magnitude; and (3) matrix challenges, as blood and blood collection containers contain molecular amplification inhibitors such as hemoglobin and SPS.



We developed Blood2Bac™, a novel method for **species agnostic ultrahigh enrichment** (UHE) of bacteria directly from whole blood that depletes human DNA by 8-10 orders of magnitude. Blood2Bac is followed by whole genome sequencing (WGS) on an Oxford Nanopore Technologies sequencing device for rapid data generation. WGS data is analyzed with Keynome® ID, our algorithm for species pathogen identification in mixed human/pathogen samples, and Keynome® gAST (genomic antibiotic susceptibility testing), our machine learning algorithm for determining AST from genomic sequences to accurately predict resistance. With an end-to-end **turnaround time of 8 hours**, we demonstrate comprehensive capabilities for whole genome recovery, species identification, and AST determination direct from blood at low input concentrations.

## Bacterial Whole Genome Recovery From Blood

Contrived whole blood samples with bacterial strains spiked in at single digit CFU/mL input concentrations were processed with Blood2Bac. Examination of the ONT WGS data demonstrates ultra-high enrichment of bacterial reads relative to human reads, and near complete genome recovery. Across the vast majority of all tested species (subset shown below, Gram positive and Gram negative), the WGS data aligned back to the spike-in strain's genome assembly revealed near complete genome recovery. These data show consistency across multiple replicate spike-ins demonstrating robustness in genome recovery.



## Agnostic Species ID Tested on >60 Bacterial Species

Over 60 bacterial species have been tested with Blood2Bac technology. The majority have demonstrated an **analytical sensitivity at ≤2 CFU/mL** input concentrations and ultra-high enrichment of bacterial to human reads in WGS data.

Gram Positive Species	ID Analytical Sens (CFU/mL)	Bacterial / Human DNA	Gram Positive Species (cont)	ID Analytical Sens (CFU/mL)	Bacterial / Human DNA
<i>Bacillus cereus</i>	≤ 2	29.6	<i>Streptococcus salivarius</i>	≤ 2	1.2
<i>Clostridium perfringens</i>	4	1.5	<i>Streptococcus sanguinis</i>	≤ 2	5.3
<i>Enterococcus avium</i>	≤ 2	73.1	<b>Gram Negative Species</b>		
<i>Enterococcus casseliflavus</i>	≤ 2	1.2	<i>Acinetobacter baumannii</i>	3	52.1
<i>Enterococcus faecalis</i>	≤ 2	76.9	<i>Acinetobacter ursingii</i>	≤ 2	21.9
<i>Enterococcus faecium</i>	≤ 2	27.2	<i>Aggregatibacter aphrophilus</i>	3	7.4
<i>Enterococcus gallinarum</i>	≤ 2	7.0	<i>Bacteroides fragilis</i>	≤ 2	0.7
<i>Enterococcus raffinosus</i>	≤ 2	7.9	<i>Bacteroides vulgatus</i>	≤ 2	2.1
<i>Lactobacillus rhamnosus</i>	≤ 2	4.6	<i>Campylobacter coli</i>	3	45.7
<i>Listeria monocytogenes</i>	≤ 2	59.2	<i>Campylobacter jejuni</i>	7	106.1
<i>Propionibacterium acnes</i>	5	0.3	<i>Cardiobacterium hominis</i>	≤ 2	10.9
<i>Staphylococcus aureus</i>	≤ 2	5.0	<i>Citrobacter freundii</i>	≤ 2	111.0
<i>Staphylococcus capitis</i>	≤ 2	4.6	<i>Citrobacter koseri</i>	≤ 2	19.2
<i>Staphylococcus caprae</i>	4	0.9	<i>Eikenella corrodens</i>	3	5.7
<i>Staphylococcus epidermidis</i>	2	9.2	<i>Enterobacter cloacae</i>	≤ 2	7.9
<i>Staphylococcus haemolyticus</i>	≤ 2	14.3	<i>Escherichia coli</i>	≤ 2	61.0
<i>Staphylococcus hominis</i>	3	4.7	<i>Haemophilus influenzae</i>	≤ 2	61.6
<i>Staphylococcus lugdunensis</i>	≤ 2	1.5	<i>Klebsiella aerogenes</i>	≤ 2	5.0
<i>Staphylococcus saprophyticus</i>	≤ 2	20.5	<i>Klebsiella oxytoca</i>	≤ 2	7.9
<i>Staphylococcus simulans</i>	8	1.2	<i>Klebsiella pneumoniae</i>	≤ 2	16.0
<i>Staphylococcus warneri</i>	≤ 2	34.2	<i>Morganella morganii</i>	≤ 2	109.7
<i>Streptococcus agalactiae</i>	≤ 2	29.5	<i>Pantoea agglomerans</i>	7	105.4
<i>Streptococcus anginosus</i>	≤ 2	19.3	<i>Pasteurella multocida</i>	≤ 2	17.1
<i>Streptococcus constellatus</i>	≤ 2	5.2	<i>Proteus mirabilis</i>	≤ 2	36.2
<i>Streptococcus dysgalactiae</i>	5	2.0	<i>Pseudomonas aeruginosa</i>	≤ 2	17.9
<i>Streptococcus intermedius</i>	≤ 2	18.4	<i>Pseudomonas putida</i>	6	0.9
<i>Streptococcus mutans</i>	4	0.5	<i>Raoultella ornithinolytica</i>	≤ 2	35.6
<i>Streptococcus oralis</i>	≤ 2	26.6	<i>Salmonella enterica</i>	≤ 2	16.0
<i>Streptococcus parasanguinis</i>	≤ 2	8.2	<i>Serratia liquefaciens</i>	≤ 2	5.5
<i>Streptococcus pneumoniae</i>	≤ 2	27.1	<i>Serratia marcescens</i>	≤ 2	4.3
<i>Streptococcus pyogenes</i>	≤ 2	5.2	<i>Stenotrophomonas maltophilia</i>	5	0.9

## Blinded Test Panel with CARB-X Demonstrates ID Capabilities

As part of a test of Blood2Bac technology, 40 blinded bacterial strains were sent to DZD from the organization CARB-X. Strains were spiked into whole blood at 6-12 CFU/mL, processed with Blood2Bac + Keynome ID. Keynome ID correctly identified 39/40 strains from blood (97.5%). In each case, **a single species ID call was made with no false positives**. 1 sample did not pass threshold for a qualified Keynome ID call. The sample was 1 of 10 designated as part of a more challenging set of strains designated by CARB-X.

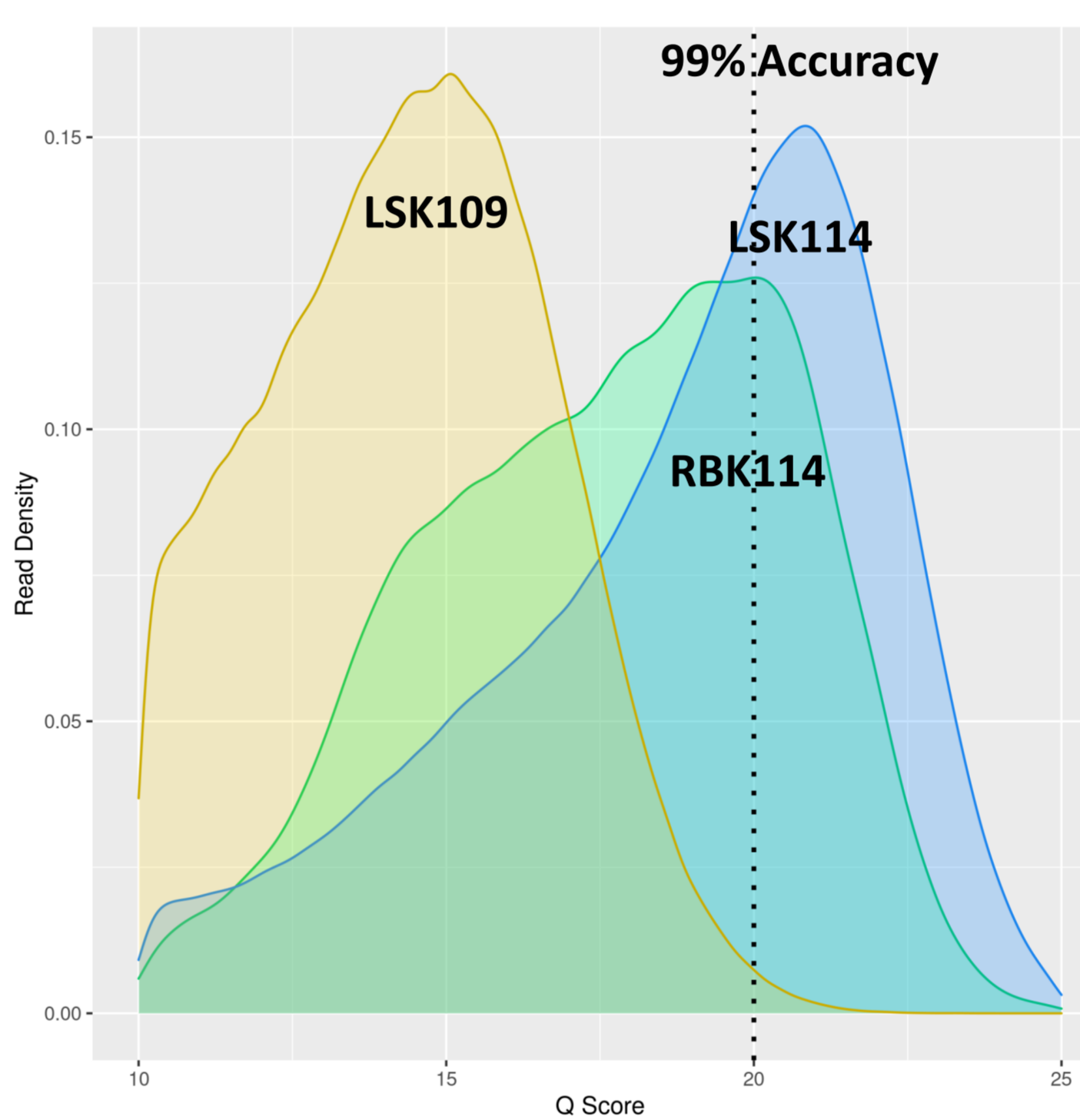
CARB-X Species ID	DZD Keynome ID	Correct
<i>E. faecalis</i>	<i>E. faecalis</i>	✓
<i>E. faecalis</i>	<i>E. faecalis</i>	✓
<i>E. faecalis</i>	<i>E. faecalis</i>	✓
<i>E. faecium</i>	<i>E. faecium</i>	✓
<i>E. faecium</i>	<i>E. faecium</i>	✓
<i>E. faecium</i>	<i>E. faecium</i>	✓
<i>S. aureus, MRSA</i>	<i>S. aureus, MRSA</i>	✓
<i>S. aureus, MSSA</i>	<i>S. aureus, MSSA</i>	✓
<i>S. aureus, MRSA</i>	<i>S. aureus, MRSA</i>	✓
<i>S. aureus, MRSA</i>	<i>S. aureus, MRSA</i>	✓
<i>S. pneumoniae</i>	<i>S. pneumoniae</i>	✓
<i>E. cloacae</i>	<i>E. cloacae</i> complex	✓
<i>E. cloacae</i>	<i>E. cloacae</i> complex	✓
<i>E. cloacae</i>	<i>E. cloacae</i> complex	✓
<i>E. coli</i>	<i>E. coli</i>	✓
<i>E. coli</i>	<i>E. coli</i>	✓
<i>E. coli</i>	<i>E. coli</i>	✓
<i>E. coli</i>	<i>E. coli</i>	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓

CARB-X Species ID	DZD Keynome ID	Correct
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>A. baumannii</i>	<i>A. baumannii</i> complex	✓
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	✓
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	✓
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	✓
<i>S. maltophilia</i>	<i>S. maltophilia</i>	✓
<i>S. maltophilia</i>	<i>S. maltophilia</i>	✓
<i>C. freundii</i>		X
<i>C. koseri</i>	<i>C. koseri</i>	✓
<i>S. epidermidis</i>	<i>S. epidermidis</i>	✓
<i>K. pneumoniae</i>	<i>K. pneumoniae</i> complex	✓
<i>S. pyogenes</i>	<i>S. pyogenes</i>	✓

Challenge Group

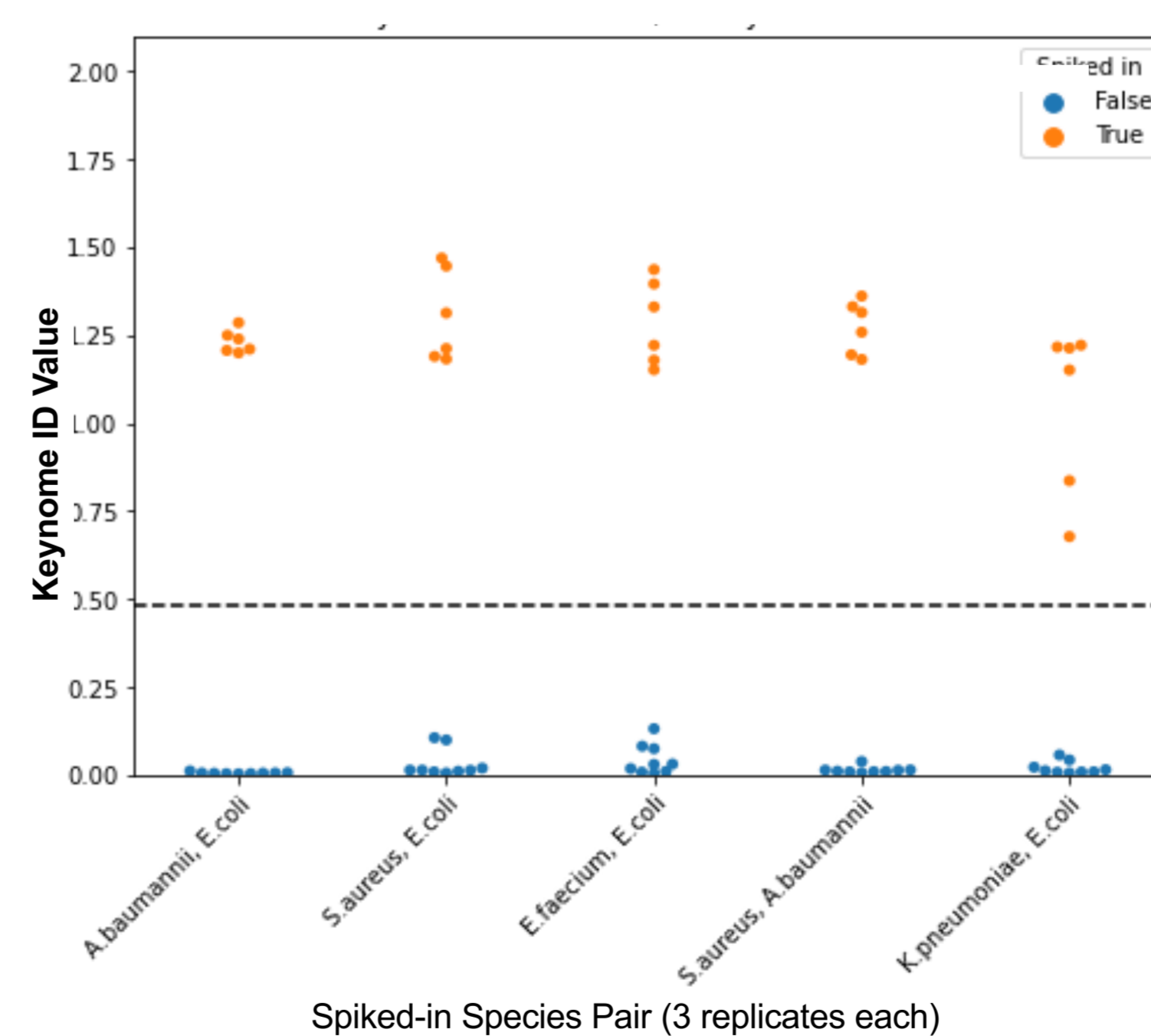
## Increased Sequencing Accuracy with ONT Kit14

After enrichment and amplification of bacterial DNA from whole with Blood2Bac, the output is sequenced on an ONT sequencing device. We compared the sequencing accuracy of preparing a Blood2Bac sample with with LSK109 and sequencing on R9.4 flowcell (orange), and preparing with LSK114 or and RBK114 protocol and sequencing on R10.4 flowcells (blue). Kit 14 data show marked improvement in data with median simplex raw read accuracy of >99% on Kit 14.



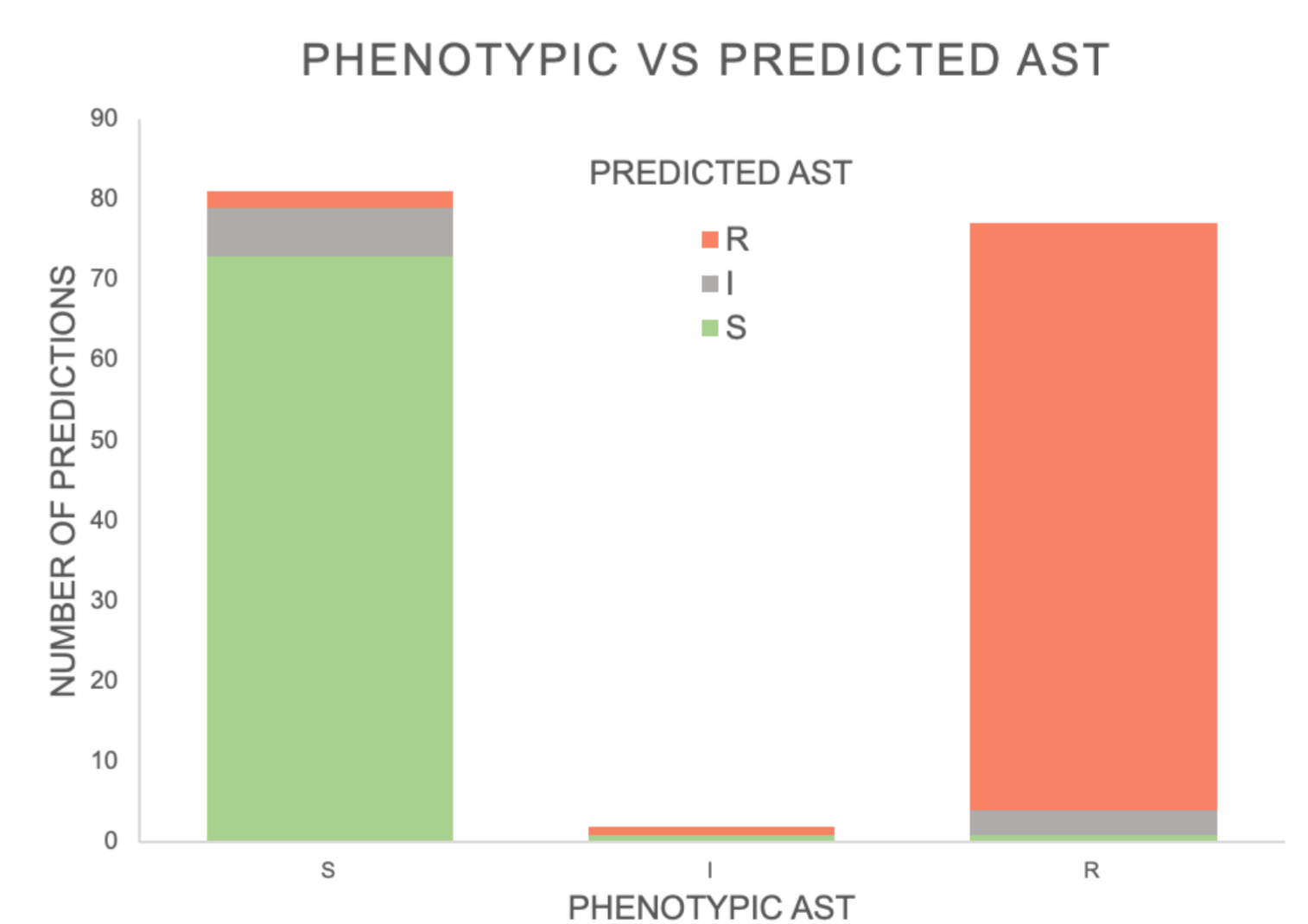
## Polymicrobial Challenge

Keynome ID was challenged with 5 polymicrobial species mixtures, 3 replicates each, spiked in at 2-13 CFU/mL and processed with Blood2Bac + Keynome ID. Across all 15 (5 x 3) samples, Keynome ID identified both species in all samples and called no false positives. The large gap in Keynome ID values between spiked-in species (orange) and other species (blue) demonstrates **high signal to noise** ratio in the WGS data, enabling low rates of false positive calls in the system.



## Keynome gAST: Direct-From-Blood Genomic AST

We tested the ability of Keynome gAST to determine antibiotic susceptibility profiles of pathogens directly from blood. 50 bacterial strains across 6 Gram positive and Gram negative species were spiked into whole blood at 5.5 - 21.5 CFU/mL and processed with Blood2Bac, sequenced on R10.4 PrometION flowcells, and analyzed with Keynome ID and Keynome gAST. 160 g-AST predictions were compared to phenotypic AST measurements for each strain. Results demonstrated **high accuracy** with Very Major Error Rate: 1.3%, Major Error Rate: 2.5%, and Categorical Agreement: 91.3%.



## Conclusions

These results demonstrate the ability of the Blood2Bac assay paired with ONT sequencing to identify bacterial pathogens and determine antibiotic susceptibility directly from blood within 8 hours, making it an exciting prospect for rapid and comprehensive BSI diagnosis. Recovery of whole genomes of pathogens directly from blood can enable other downstream applications, such as surveillance of hospital acquired infections and detection of novel genomic elements.