Clinical Evaluation of a Sequencing-based Diagnostic for Bacterial & Fungal ID & AST Directly from Patient Blood Samples

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Background

- Early pathogen ID and targeted treatment are key to reducing bloodstream infection (BSI) morbidity and mortality.
- Current diagnostics rely on culture which takes 1-2 days for ID and longer for antimicrobial susceptibility testing (AST) results, or molecular assays which have limited panels, few resistance markers, and high false positive rates.
- The Day Zero Diagnostic (DZD) system aims to deliver results in ~8 hours, uses ultra-high enrichment of microbial DNA directly from blood, whole-genome sequencing, and a predictive AST machine-learning algorithm trained on 75,000+ pairs of whole pathogen genome and phenotypic inputs to identify a broad range of species and pathogen/drug combinations.
- **Clinical Study Objective: To compare the performance of a first-in-kind comprehensive ID and predictive AST genomic direct-from-blood diagnostic assay to hospital microbiology lab phenotypic ID/AST results from blood cultures.**

Study Methods

- 2 IRB approved observational studies enrolled subjects with suspicion of BSI
- 3 EDs (RAPPID) and 1 ICU/inpatient unit (BRABIT) in 4 Boston area hospitals
- Samples: whole blood in SPS vacutainers; 10mL processed at DZD
- Processed with proprietary Pathovate™ process for ultra-high enrichment (UHE) of microbial DNA directly from blood with near complete genome recovery
- Sequencing performed on an Oxford Nanopore platform
- Sequencing data were analyzed by Keynome® algorithms to determine pathogen ID and predict genomic AST (gAST) profiles
- Results were compared to ID and AST standard-of-care methods

Keynome ID Species Panel (29 pathogens)

Gram-negative bacteria
- *A. baumannii* complex
- *C. freundii* complex
- *E. cloacae* complex
- *E. coli**
- *K. aerogenes*
- *K. oxytoca*
- *K. pneumoniae* complex*
- *M. morganii*
- *P. mirabilis*
- *P. aeruginosa**
- *P. putida*
- *S. marcescens**

Gram-positive bacteria
- *B. cereus* group
- *E. faecalis*
- *E. faecium**
- *S. aureus**
- *S. lugdunensis*
- *S. agalactiae**
- *S. anginosus* group
- *S. dysgalactiae* group
- *S. mutans*
- *S. mitis* group
- *S. pyogenes**
- *S. sanguinis*

Fungi
- *Candida albicans*
- *Candida auris*
- *Candida glabrata*
- *Candida krusei*
- *Candida tropicalis*

* Indicates observed species

Results

Keynome ID Performance

- 225 samples had blood culture and Keynome ID results (6525 calls); 96.9% (218/225) drawn simultaneously with clinical culture
- 37 (16%) blood culture positive samples
  - 20/37 BC positive species were on-panel
  - 15/37 BC positives were off-panel because they are common skin flora/oral commensals (*S. capitis, S. epidermidis, S. haemolyticus, S. hominis, S. pettenkoferi, S. salivarius, A. oris/viscosus, D. nishinomiyaensis*)
  - 2/37 BC positives were off-panel because the targets are under development (*S. pneumoniae, H. influenzae*)
- Per on-panel species performance had 80.0% sensitivity, 99.9% specificity, and 99.8% agreement with clinical culture (**Table 1**).
- Per-sample performance demonstrated 80.0% sensitivity, 96.6% specificity, and 95.1% agreement with clinical culture (**Table 2**).
- Of the false positives, 2 were potential transient bacteremia and 5 were likely lab contamination.

Table 1: Keynome ID panel performance summary across 225 patient samples.

Species	TP	TN	FN	FP	Sensitivity (sample counts)	Specificity (sample counts)	PPV	NPV	Overall Agreement
All	**16**	**6498**	**4**	**7**	**80.0% (16/20)**	**99.9% (6498/6505)**	**69.6%**	**99.9%**	**99.8%**
Bacillus cereus group	0	224	0	1	-	99.6% (224/225)	0.0%	100.0%	99.6%
Enterococcus faecium	1	223	1	0	50.0% (1/2)	100.0% (223/223)	100.0%	99.6%	99.6%
Escherichia coli	4	218	1	2	80.0% (4/5)	99.1% (218/220)	66.7%	99.5%	98.7%
Klebsiella pneumoniae complex	2	222	1	0	66.7% (2/3)	100.0% (222/222)	100.0%	99.6%	99.6%
Pseudomonas aeruginosa group	1	224	0	0	100.0% (1/1)	100.0% (224/224)	100.0%	100.0%	99.1%
Serratia marcescens	1	224	0	0	100.0% (1/1)	100.0% (224/224)	100.0%	100.0%	100.0%
Staphylococcus aureus	5	217	0	3	100.0% (5/5)	98.6% (217/220)	62.5%	100.0%	98.7%
Streptococcus agalactiae	1	223	1	0	50.0% (1/2)	100.0% (223/223)	100.0%	99.6%	99.6%
Streptococcus mutans	0	224	0	1	-	99.6% (224/225)	0.0%	100.0%	99.6%
Streptococcus pyogenes	1	224	0	0	100.0% (1/1)	100.0% (224/224)	100.0%	100.0%	100.0%

* Additional 19 species on-panel not shown were blood culture and Keynome ID negative

Table 2: Keynome ID per patient performance

- Sensitivity: 80.0% (16/20)
- Specificity: 96.6% (199/206)
- Positive Predictive Value: 69.6%
- Negative Predictive Value: 98.0%
- Overall agreement: 95.1%

Pathovate \ Blood Culture	Positive	Negative	Total
Positive	16	7*	**23**
Negative	4*	199	**203**
Total	**20**	**206**	**226***

*There were 225 patient samples; rappid-bwh-141 was FN for *E. faecalis* and FP for *S. aureus.*

Keynome *g*AST Performance

- With a median (IQR) coverage of 96.7% (92.8-99.4%), Pathovate UHE provides high genome recovery to enable breadth of coverage assessment (Keynome ID) of pathogen in a clinical blood sample.
- Of the positive samples that qualified for AST analysis (13/16), the predicted AST results had 92.3% agreement with phenotypic AST from blood culture (**Table 3**).
- DZD's machine learning approach to genomic AST compares to standard of care phenotypic AST methods.

Table 3: KgAST performance summary

	Performance
# Phenotypic AST results (S | I | R)	**65** (39 | 4 | 22)
# species	**4**
# drugs	**14**
Very Major Error rate	**0.0%** (0.0% - 14.9%)
Major Error rate	**2.6%** (0.5% - 13.2%)
Minor Error rate	**6.2%** (2.4% - 14.8%)
Categorical Agreement	**92.3%** (82.2% - 96.7%)

Error rates (with 95% confidence intervals) and counts for predictive AST models

Limitations

- Current Pathovate version has 29 pathogens
- Enhancements to Pathovate and growing reference library will allow additional pathogens to be added in the future
- Present Pathovate process is manual, which introduces opportunity for contamination. A new closed-system, automated prototype will speed in-hospital processing and eliminate manual steps

Conclusions

- **First clinical demonstration of a novel system that can provide comprehensive ID & AST results with whole genome recovery directly from blood**
- **ID results demonstrate 99.8% agreement with blood culture and 92.3% categorical agreement with phenotypic AST**
- **Assay has potential to reduce speed to diagnosis, thus facilitating targeted therapy, improved outcomes, and reduced antimicrobial resistance.**

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