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# Rapid Species ID and AST Direct from Whole Blood: Initial Results from a Feasibility Study in Patients with Suspected Blood Stream Infection

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## Background

Species ID and AST direct from blood, without the need for culture, remains an elusive goal of rapid diagnostics. Current genomic approaches for pathogen detection are challenged by the billion-fold greater quantity of host DNA relative to pathogen DNA, the presence of inhibitors to molecular techniques, and cell-free DNA from spurious sources. Day Zero Diagnostics (DZD) has developed a direct-from-blood, sample-to-answer assay to deliver comprehensive ID and AST directly from clinical samples in 6-8 hours versus 2-4 days of standard microbiological methods. DZD's Pathovate™ enrichment process recovers clinical pathogen genomes for rapid, whole genome sequencing. DZD has also developed novel algorithms (Keynome) for bacterial or fungal ID and AST prediction using machine learning. We previously demonstrated ID and AST performance on contrived blood samples spiked with single digit CFU/mL pathogen concentrations. Here we present results from the feasibility phase of an ongoing clinical study.

## Study Methods

Study participants (117 subjects enrolled, 87 samples were eligible for processing and analysis) were prospectively enrolled from the emergency department (RAPPID) or inpatient units (BRABIT) of four Boston-area hospitals with IRB approval. Whole blood was collected in SPS vacutainers and transported to DZD, where 10mLs of blood were processed with Blood2Bac and sequenced on the Oxford Nanopore Technologies (ONT) platform. Sequencing data was analyzed by Keynome to determine the presence/absence of pathogens and their AST profiles. Performance was compared to study site clinical microbiology lab results using blood cultures collected within 24 hours of the research draw.

Table 1: Study demographics for both ED and inpatient

| Characteristic | RAPPID (n=80) | BRABIT (n=7) |
|---|---|---|
| **Age**, median (IQR), years | 67 (56 – 75) | 50 (36 – 56) |
| **LOS**, median (IQR), days | 7.8 (5.1 – 13.1) | - |
| **Sex**, n (%)<br>Male<br>Female | 47 (59)<br>33 (41) | 5 (71)<br>2 (29) |
| **Race**, n (%)<br>White<br>Non-White | 60 (75)<br>20 (25) | 5 (71)<br>2 (29) |
| **Ethnicity**, n (%)<br>Hispanic or Latino<br>Not Hispanic or Latino | 5 (7)<br>71 (93) | 1 (14)<br>6 (86) |
| **Living Situation**, n (%)<br>Home<br>Facility<br>Homeless | 67 (88)<br>8 (11)<br>1 (1) | - |

**Pathovate™ + Keynome®**
**compared to standard microbiology blood culture**

117 total subjects enrolled
- 12 no consent
- 10 insufficient blood volume
- 4 blood culture canceled
- 4 invalid results

87 samples processed with Pathovate + Keynome

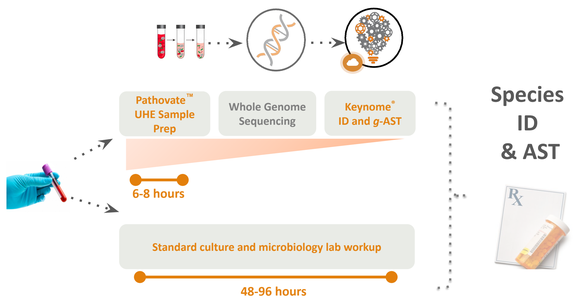


Table 2: Pathovate recovers whole genomes from clinical blood samples

| Sample ID | Enrollment BCx & Collection Delta | KID (KID value) | Coverage 1x |
|---|---|---|---|
| rappid-mgh-312 | ***Streptococcus agalactiae* (0:00)** | ***Streptococcus agalactiae* (0.97)** | 97.1% |
| rappid-mgh-338 | ***Klebsiella pneumoniae* (0:00)** | ***Klebsiella pneumoniae* (0.97)** | 95.0% |
| rappid-bidmc-49 | ***Serratia marcescens* (0:00)** | ***Serratia marcescens* (0.35)** | 32.7% |
| rappid-bwh-163 | ***Escherichia coli* (0:00)** | ***Escherichia coli* (0.98)** | 98.5% |
| rappid-bwh-164 | ***Enterococcus faecium* (0:00)** | ***Enterococcus faecium* (0.62)** | 57.4% |
| rappid-mgh-376 | ***Klebsiella pneumoniae* (0:00)** | ***Klebsiella pneumoniae* (0.94)** | 96.3% |
| brabit-255 | ***Staphylococcus aureus* (19:40)** | ***Staphylococcus aureus* (1.39)** | 100% |
| brabit-259 | ***Staphylococcus aureus* (17:11)** | ***Staphylococcus aureus* (0.64)** | 75.5% |

## Results

### Keynome ID Performance

- Pathovate UHE provides high genome recovery to enable breadth of coverage assessment (Keynome ID) of pathogen in a clinical blood sample.
- Six distinct on-panel species were identified across 10 positive samples.
- Blood culture and DZD results demonstrated an overall agreement of 99.9%, with an 80.0% sensitivity and 99.9% specificity (Table 3).

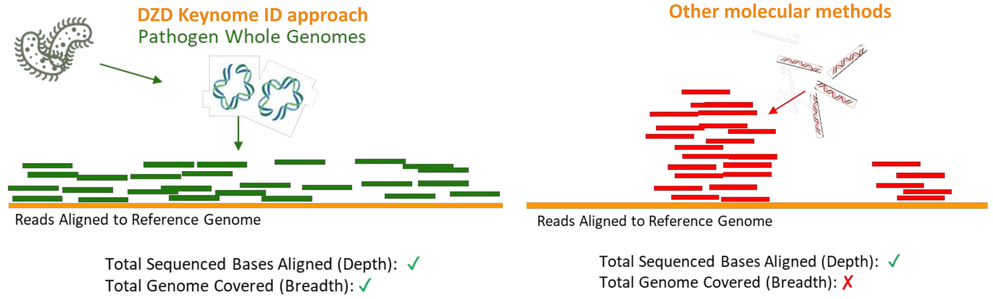


Table 3: Keynome ID performance summary across 117 patient samples.

| Species | TP | TN | FN | FP | Sensitivity (sample counts) | Specificity (sample counts) | PPV | NPV | Overall Agreement |
|---|---|---|---|---|---|---|---|---|---|
| All | 8 | 2511 | 2 | 2 | 80.0% (8/10) | 99.9% (2511/2513) | 80.0% | 99.9% | 99.8% |
| *Enterococcus faecium* | 1 | 85 | 1 | 0 | 50.0% (1/2) | 100% (85/85) | 100% | 98.8% | 98.9% |
| *Escherichia coli* | 1 | 86 | 0 | 0 | 100% (1/1) | 100% (86/86) | 100% | 100% | 100% |
| *Klebsiella pneumoniae* complex | 2 | 84 | 1 | 0 | 66.7% (2/3) | 100% (84/84) | 100% | 98.8% | 98.9% |
| *Serratia marcescens* | 1 | 86 | 0 | 0 | 100% (1/1) | 100% (86/86) | 100% | 100% | 100% |
| *Staphylococcus aureus* | 2 | 83 | 0 | 2 | 100% (2/2) | 97.6% (83/85) | 50.0% | 100% | 97.7% |
| *Streptococcus agalactiae* | 1 | 86 | 0 | 0 | 100% (1/1) | 100% (86/86) | 100% | 100% | 100% |

### Keynome *g*AST Performance

- Keynome *g*AST pipeline includes over 200 AST prediction models.
- Out of the positive samples that qualified for AST analysis (5/8), the predicted AST results had 100% agreement with phenotypic AST from blood culture (Table 4).
- DZD's machine learning approach to genomic AST compares to standard of care phenotypic AST methods (e.g. VITEK 2, Kirby-Bauer).

Table 4: *Kg*AST performance summary

| | High confidence panel | Extended R&D panel |
|---|---|---|
| **# samples** | 5 | 5 |
| **# Phenotypic AST results (S \| I \| R)** | 26 (17 \| 1 \| 8) | 26 (24 \| 1 \| 1) |
| **# species** | 4 | 4 |
| **# drugs** | 13 | 15 |
| **vME rate** | 0.0% (0.0-32.4%) | 0.0% (0.0-79.4%) |
| **ME rate** | 0.0% (0.0-18.4%) | 0.0% (0.0-13.8%) |
| **mE rate** | 0% (0.0-12.9%) | 3.85% (0.7-18.9%) |
| **CA** | 100% (87.1-100%) | 96.15% (81.1-99.3%) |

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

Table 5: *Kg*AST predictions compared to standard testing

| Antibiotic | Standard Testing | *Kg*AST |
|---|---|---|
| **rappid-mgh-338 *K. pneumoniae*** | | |
| Amikacin | S | - |
| Ampicillin | R | - |
| Aztreonam | - | S |
| Cefazolin | S | - |
| Cefepime | S | - |
| Ceftriaxone | S | S |
| Ciprofloxacin | S | - |
| Ertapenem | S | - |
| Gentamicin | S | S |
| Imipenem | - | S |
| Levofloxacin | S | - |
| Meropenem | S | - |
| Piperacillin-tazobactam | S | - |
| Tetracycline | S | - |
| Tobramycin | S | S |
| Trimethoprim/Sulfa | S | S |
| **rappid-mgh-376 *K. pneumoniae*** | | |
| Amikacin | S | - |
| Ampicillin | R | - |
| Aztreonam | - | S |
| Cefepime | S | - |
| Ceftriaxone | S | S |
| Ciprofloxacin | S | - |
| Ertapenem | S | S |
| Gentamicin | S | S |
| Imipenem | - | S |
| Levofloxacin | S | - |
| Meropenem | S | - |
| Nitrofurantoin | I | - |
| Piperacillin-tazobactam | S | - |
| Tetracycline | S | - |
| Tobramycin | S | S |
| Trimethoprim/Sulfa | R | R |
| **brabit-255 *S. aureus*** | | |
| Ceftaroline | S | - |
| Ciprofloxacin | - | S |
| Clindamycin | S | S |
| Daptomycin | S | - |
| Doxycycline | - | S |
| Erythromycin | R | R |
| Gentamicin | - | S |
| Levofloxacin | S | S |
| Moxifloxacin | - | S |
| Oxacillin | R | R |
| Penicillin | R | - |
| Tetracycline | S | S |
| Trimethoprim/Sulfa | S | - |
| Vancomycin | S | - |

| Antibiotic | Standard Testing | *Kg*AST |
|---|---|---|
| **rappid-bwh-163 *E. coli*** | | |
| Amikacin | S | - |
| Amoxicillin + Clavulanate | S | - |
| Ampicillin | R | R |
| Aztreonam | - | S |
| Cefepime | S | - |
| Cefotaxime | - | I |
| Cefoxitin | S | - |
| Ceftazidime | S | S |
| Ceftriaxone | S | S |
| Ciprofloxacin | R | R |
| Ertapenem | S | - |
| Gentamicin | S | S |
| Imipenem | - | S |
| Levofloxacin | R | R |
| Meropenem | S | - |
| Piperacillin-tazobactam | S | - |
| Tetracycline | R | R |
| Tobramycin | S | S |
| Trimethoprim/Sulfa | R | R |
| **rappid-mgh-312 *S. agalactiae*** | | |
| Ceftriaxone | S | - |
| Clindamycin | S | S |
| Erythromycin | I | I |
| Penicillin G | S | - |
| Vancomycin | S | - |

## Conclusions

These preliminary data suggest that the DZD process can provide highly accurate ID and AST results directly from blood in patients with suspected BSI. To our knowledge, these results mark the first demonstration of whole genome recovery and comprehensive ID/AST directly from patient blood samples, highlighting the potential to improve outcomes and time to result.

## Acknowledgements

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