Saturday
22 June 2019
CPHM-882

One Step Closer to *Enterobacteriaceae* ID/AST Direct from Blood: Evaluating Pre-Process Incubation Times

S. Giddins[1], I. Yushkevich[1], M. Fuchs[2] , A. Irwin[1], S. Metzger[2], C. Price[1]

[1] Denver Health and Hospital Authority, Denver, CO; [2] Accelerate Diagnostics, Inc., Tucson, AZ

DENVER HEALTH est. 1860 FOR LIFE'S JOURNEY

Sara Giddins
DHHA
Phone: (303) 602-2378
Sara.Giddins@dhha.org

Amended Abstract

Background: Detection of bacteria directly from blood in septic patients is a critical step to improving patient morbidity/mortality and antibiotic stewardship. Low bacterial loads in bacteremic blood present a distinct obstacle, however. To overcome this diagnostic challenge, a short incubation step is needed to increase bacterial cell numbers prior to performing identification (ID) and antimicrobial susceptibility testing (AST) using an Accelerate Pheno™ system (AXDX) custom assay. As part of assay optimization, several pre-process incubation times were tested using contrived, low concentration bacteremic blood samples.

Methods: Experiments with ATCC® and CDC *Enterobacteriaceae* strains were conducted using 10 mL donor blood in 30 mL lytic growth medium and internally-developed sample preparation procedures. After 3, 4, 4.5, or 5 h incubation at 35-37°C, processed samples were tested using AXDX with a high-efficiency custom assay for bacterial detection. Initial inocula and final sample concentrations were determined by quantitative plating. For ID, bacterial cells were manually counted in fluorescent green images: 1 site/flowcell (FC) in 10 FCs to obtain an average number of bacterial cells/FC. For AST, automated growing clone (GC) analysis was used to determine bacterial concentration in growth FCs.

Results: Starting inocula ranged from 0.8 to 1.7 CFU/mL in 10 mL donor blood. Visual analysis of ID channels showed bacterial cells in singles, clumps, and chains, with many clumps of 20 or more cells. See Table 1 for results of manual cell counts for ID and automated GC analysis for AST.

Conclusions: AXDX automated algorithms work with as few as 150 cells/FC for ID and 240 - 3,120 GCs/FC for AST. In the custom assay, multiple FCs will be dedicated to growth and each antibiotic concentration, facilitating automated AST results with < 240 GCs/FC. At a starting concentration of < 2 CFU/mL, these contrived experiments adequately represented a low clinical bacterial load of 1 - 10 CFU/mL. Pre-process incubation at 3 h did not consistently yield the target number of GCs, and over-saturation occurred at 5 h. The ideal incubation time was determined to be 4 to 4.5 h for automated AST and confident ID. This investigation is an important step in making direct from blood ID/AST attainable.

Methods

Experiment Steps (see Figure 1):

1. Prepare inoculum suspension of *Enterobacteriaceae* isolate (*E. coli* ATCC® 25922 n=8, *K. pneumoniae* ATCC® 700603 n=8, *K. pneumoniae* CDC strain AR-0016 n=4, *E. aerogenes* ATCC® 13048 n=6, and *E. cloacae* CDC strain AR-0073 n=8)
2. Seed donor whole blood with known concentration of inoculum and add media (quantitatively plate to confirm); target concentration = 1 to 10 CFU/mL in 10 mL whole blood
3. Incubate experiment for 3, 4, 4.5 or 5 h (35°C, shaking at 120 rpm)
4. Perform rapid blood sample processing (manual spin/re-suspend steps in various buffers to remove human cells and concentrate the sample down to 0.9 mL)
5. Quantitatively plate final sample to confirm concentration
6. Load sample onto Accelerate Pheno™ system (AXDX) for automated assay
7. AXDX loads flowcells with sample (glass-bottomed microfluidic channels in which bacteria are drawn to the surface for imaging)
8. AXDX stains cells in ID channels with propidium iodide and takes images via fluorescence microscopy

Methods

Experiment Steps continued:

9. Manually analyze ID images and count bacterial cells in representative sites and experiments (site = a small division of each FC equivalent to one microscopic field of view and one image)
10. Review automated growth analysis in AST flowcells (AXDX tracks growing clones using time-lapse dark-field imaging)
11. Calculate cells per ID flowcell and average growing clones per growth flowcell for each condition (Table 1)
12. Compare results to known AXDX automated algorithm capabilities (Table 1)

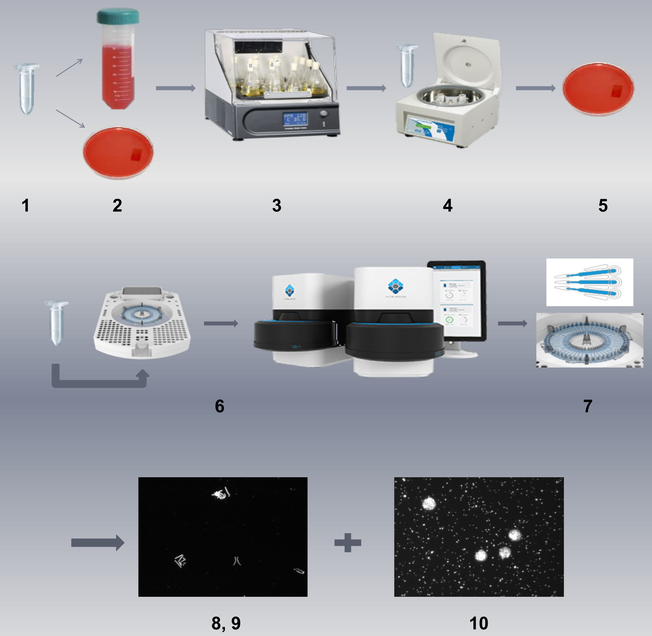

Figure 1: Experiment Steps

Results

Table 1

	Pre-Process Incubation Time				Automated Algorithm Parameters
	3 hrs (n=8)	4 hrs (n=12)	4.5 hrs (n=4)	5 hrs (n=4)	n/a
Starting Inoculum CFU/mL	1.2 – 1.4	0.8 – 1.7	1.0	1.2-1.7	n/a
Final Sample CFU/mL	$4.5 \times 10^3 – 1.2 \times 10^4$	$1.3 – 7.0 \times 10^4$	$1.0 – 2.7 \times 10^5$	$4.3 – 8.2 \times 10^5$	n/a
ID or Detection*: Cells/FC	70 – < 500	800 – 5,700	> 4,000	> 4,000	≥ 150
AST or Growth: GCs/FC	2 – 37	40 – 1,428	767 – 2,338	82 – 3,458	240-3,120

* The extensive time required for manual analysis precluded complete numeration of bacterial cells in ID flowcells, so only a representative number of experiments were analyzed.

- A total of 34 *Enterobacteriaceae* experiments were run; data from 6 *E. cloacae* runs were excluded due to high debris levels interfering with automated growth analysis.
- Initial contrived sample bacterial concentrations ranged from 0.8 to 1.7 CFU/mL in 10 mL whole blood, simulating low bacterial load bacteremic blood (Table 1).
- 4 h and 4.5 h incubation prior to processing yielded sufficient numbers of bacterial cells in both ID and AST flowcells for automated results when calculated over multiple flowcells (Table 1); in the custom assay, 10 flowcells will be dedicated to each AST or growth condition.
- 3 h incubation yielded too few cells for automated ID and AST; 5 h resulted in oversaturation of AST images (Table 1).

Limitations

- Pre-process incubation time investigation was performed on contrived bacteremic samples with healthy donor blood, which may not entirely mimic bacteremic patient blood.

Conclusions

- The ideal pre-process incubation time for contrived *Enterobacteriaceae* bacteremic samples prior to processing and concentration was 4 to 4.5 hours, with the goal of enabling automated ID and AST directly from blood in a custom AXDX assay.
- Next steps toward reaching this goal include testing with antibiotics for AST, adding fluorescence *in situ* hybridization (FISH) probes for more specific ID, and incorporating more automated algorithms for analysis and result reporting.

Research reported in this abstract and poster was supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award number R01AI116993.
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.