Fully Automated Preparation of Positive Blood Cultures for Rapid Organism Identification Using MALDI-ToF Analysis

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C. Michel[1], H. Sullivan[2], C. Keyak[2], A. Campos-Alvarez[1], M. Blamires[1], R. Nyberg[1]

[1]Accelerate Diagnostics Inc., Tucson, AZ, [2]Alverno Laboratories, Hammond, IN

Carlos Michel
Accelerate Diagnostics
520-313-3901
cmichel@axdx.com

INTRODUCTION

Faster identification of pathogens is needed to improve care of bacteremic patients

- Delayed administration of antimicrobial agents to patients with bloodstream infection increases the potential for morbidity and mortality
- Rapid identification (ID) and antibiotic susceptibility testing (AST) of infectious pathogens is necessary for a maximal therapeutic impact
- A faster ID result directly from positive blood culture (PBC) can provide actionable results to optimize therapy for bacteremic patients
- MALDI-ToF ID offers a vast library of hundreds of species-level results but requires isolated colonies slowly grown from an overnight culture
- Molecular based ID tests are rapid and highly sensitive but expensive and typically provide a limited panel of ID results

The Accelerate Arc™ system and BC kit* is an automated sample preparation method for PBC samples for subsequent ID using MALDI-ToF analysis

- The Accelerate Arc system* is a scalable sample preparation device capable of running 1-8 samples simultaneously
- The Accelerate Arc BC kit* contains a disposable capsule used for in-line centrifugation and sample preparation consumables and reagents
- ***Figure 1*** shows the Accelerate Arc system* featuring two sample preparation modules, touchscreen monitor, and handheld barcode reader
- ***Figure 2*** shows simple workflow steps for completing the Accelerate Arc BC kit* workflow from sample to MALDI-ToF ID results

The Accelerate Arc BC kit* sample output can be analyzed using Bruker MBT Sepsityper® software extension on MBT-CA system

- The output from the automated Accelerate Arc system* can be used for preparation and analysis on MBT-CA system
- Arc processed PBC sample can be prepared using Direct Transfer (DT) and extended Direct Transfer (eDT) MALDI spotting procedures
- DT and eDT spot preparations can be analyzed using the MBT-CA Sepsityper® software extension, according to Instructions for Use

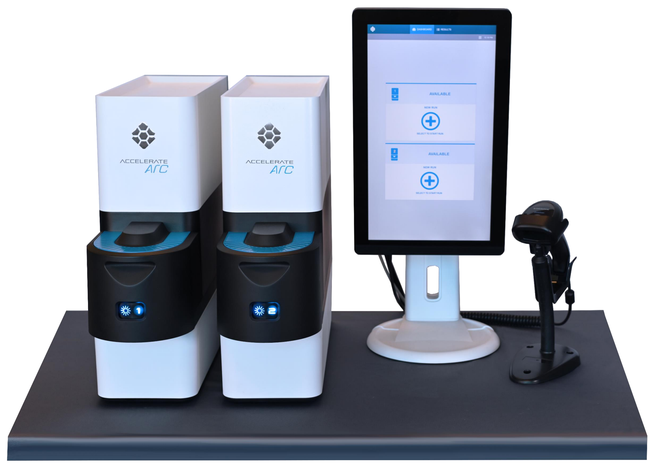
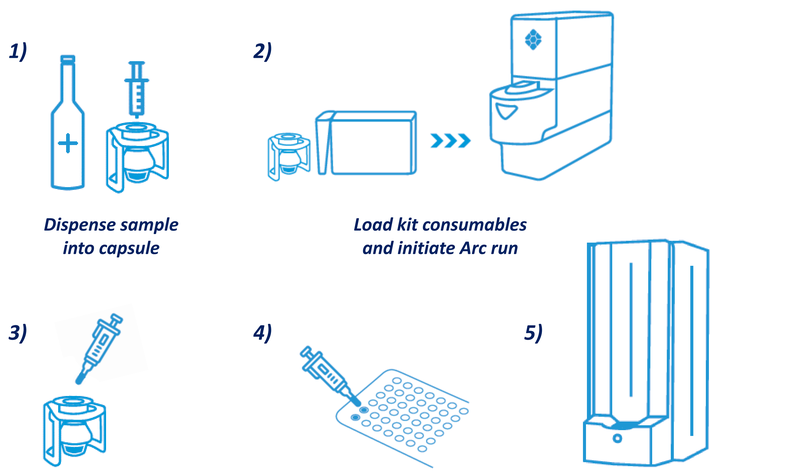


Figure 1: Accelerate Arc system* with touchscreen monitor and barcode scanner

Figure 2: Accelerate Arc BC kit* workflow for generation of fast ID from PBC sample

1. *Dispense 1.7mL (+/- 0.2mL) PBC sample into bottom of Arc capsule and re-cap*
2. *Load capsule and reagent cartridge into Arc module; close door and initiate run*
3. *After run, capsule contains processed PBC sample ready for direct transfer to MALDI plate*
4. *Prepare DT (Direct Transfer) and eDT (extended Direct Transfer) test spots according to MBT-CA workflow*
5. *Analyze using MBT Sepsityper® software extension on MBT-CA system*

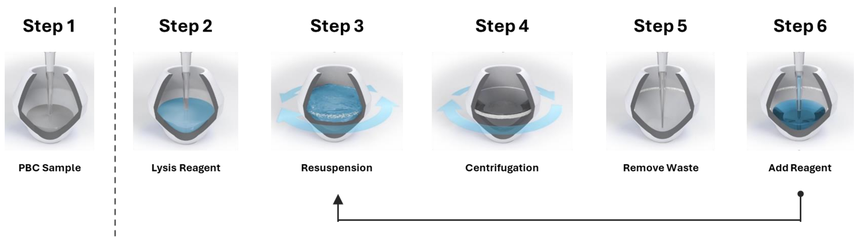


PRINCIPLES OF OPERATION

The Accelerate Arc BC kit and system* is a closed, automated sample preparation system for isolating microbial cells from a PBC sample

- The Accelerate Arc BC kit* consists of a sample capsule for loading an aliquot of PBC sample and a reagent cartridge
- Automated sample processing occurs through programmed fluid handling and repeated resuspension and centrifugation cycles
- Microbial cells are isolated and concentrated directly from PBC by removing blood, cellular debris, and culture media matrix components
- ***Figure 3*** outlines basic principles of operation of the Accelerate Arc BC kit* sample processing procedure
  - Steps shown to the right of the vertical dashed line represent steps which are performed inside the Accelerate Arc module
  - Step 1: Manually add PBC sample into the capsule and load capped capsule and reagent cartridge into an Arc module
  - Step 2: Reagents are added to lyse Red Blood Cells (RBC) and prepare sample for isolation and concentration of microbial cells from PBC
  - Step 3: Repeated cycles of low-speed rotation at high acceleration serve to vigorously mix sample components prior to centrifugation
  - Step 4: High speed, inline centrifugation serves to concentrate and capture microorganisms toward the equatorial region of capsule
  - Step 5: Waste, containing interfering components, is removed from the bottom of the capsule and dispensed to reagent cartridge
  - Step 6: New reagents are added for subsequent sample processing and a new cycle of resuspension and centrifugation follows

Figure 3: Accelerate Arc BC kit and system* automated sample processing procedure steps



CRITERIA FOR VALID RESULTS

Reportable ID from Accelerate Arc BC kit* and MBT-CA Sepsityper® software extension on the MBT-CA system

- A valid identification result was defined as a minimum of one reportable ID result per set of DT + eDT spot tests prepared for MBT Sepsityper® software extension analysis on the MBT-CA system
  - A reportable ID included High Confidence (≥ 1.80) and Low Confidence (1.60 - 1.79) log scores
  - Results with log scores < 1.60 are considered a non-reportable ID
- For each Arc-processed sample prepared using DT and eDT workflow and MBT Sepsityper® software extension, the final ID result was interpreted as follows according to MBT Sepsityper® labeling:
  - If both DT and eDT spots provide a reportable ID result and both ID results are identical, then the highest log score was considered the final result for that sample.
  - If only one of the DT and eDT spots provides a reportable ID result (high or low confidence), then the log score of the reportable ID will be considered the final result for that sample.
  - If both DT and eDT spots provide a reportable ID result but the ID results are not identical at the species level, then the discordant ID results are considered a final no identification result for that sample

Contrived Positive Blood Cultures

Organism Group	Reportability (High Confidence = ≥ 1.8 log(score); High+Low Confidence = ≥ 1.6 log(score))			Accuracy
	High	*High+ Low*	*No ID*	*Total*
GN (n=12 spp)	*80.6% (50/62)*	*91.9% (57/62)*	*8.1% (5/62)*	*100% (57/57)*
GP (n=8 spp)	*72.9% (27/37)*	*83.4% (31/37)*	*16.2% (6/37)*	*100% (31/31)*
All Orgs (n=20 spp)	*77.7% (77/99)*	*88.8% (88/99)*	*11.1% (88/99)*	*100% (88/88)*

Figure 4: ID Reportability and Accuracy Performance for contrived PBC samples
(Reportability per sample is defined by above criteria; each percentage shown includes the total number of samples tested per organism group, GN, GP, or All Orgs, i.e. combined; Accuracy is assessed by comparing only samples with a valid, reportable ID result to the comparator ID result from isolated colonies from culture of PBC)

GN Contrived
Citrobacter freundii (6)
Escherichia coli (6)
Pseudomonas aeruginosa (6)
Acinetobacter baumannii (5)
Enterobacter cloacae (5)
Klebsiella aerogenes (5)
Klebsiella oxytoca (5)
Klebsiella pneumoniae (5)
Proteus mirabilis (5)
Proteus vulgaris (5)
Serratia marcescens (5)
Citrobacter koseri (4)

GP Contrived
Staphylococcus aureus (6)
Staphylococcus epidermidis (6)
Enterococcus faecalis (5)
Enterococcus faecium (5)
Staphylococcus lugdunensis (4)
Streptococcus agalactiae (4)
Streptococcus pyogenes (4)
Streptococcus pneumoniae (3)

GN Fresh
Escherichia coli (8)
Bacteroides fragilis (5)
Klebsiella pneumoniae (4)
Proteus mirabilis (3)
Moraxella osloensis (2)
Pseudomonas aeruginosa (2)
Bacteroides stercoris (1)
Bacteroides uniformis (1)
Fusobacterium nucleatum (1)
Haemophilus influenzae (1)
Negativicoccus succinicivorans (1)
Neisseria gonorrhoeae (1)
Pasteurella multocida (1)

GP Fresh
Staphylococcus epidermidis (12)
Staphylococcus hominis (4)
Enterococcus faecalis (3)
Staphylococcus aureus (3)
Cutibacterium acnes (2)
Micrococcus luteus (2)
Streptococcus pneumoniae (2)
Actinomyces odontolyticus (1)
Clostridium ramosum (1)
Corynebacterium striatum (1)
Corynebacterium tuberculostearicum (1)
Dermabacter hominis (1)
Enterococcus faecium (1)
Gemella morbillorum (1)
Lactobacillus rhamnosus (1)
Microbacterium aurum (1)
Staphylococcus capitis (1)
Staphylococcus cohnii (1)
Staphylococcus pettenkoferi (1)
Staphylococcus succinus (1)
Streptococcus agalactiae (1)
Streptococcus anginosus (1)
Streptococcus constellatus (1)
Streptococcus parasanguinis (1)

Figure 6: List of organisms tested from PBC samples in both studies
(Each list includes name of organism and number of strains tested based on comparator ID from isolated colony testing from culture)

METHODS

Performance of the Accelerate Arc BC kit and system* using MBT Sepsityper® software extension on the MBT-CA system

- Two studies were conducted testing both contrived (at AXDX) and fresh (at Alverno) patient PBC samples

Contrived Positive Blood Culture Study

- Internal studies at Accelerate Diagnostics used 20 different Gram negative (GN) and Gram positive (GP) species
- In total 99 bacterial strains, 62 GN and 37 GP, were prepared and inoculated into BD BACTEC™ Aerobic PLUS blood culture bottles along with fresh human donor blood and incubated until flagging positive in the BD BACTEC™ FX system
- PBC bottles were removed at positivity (< 1h) and samples processed using the Accelerate Arc BC kit and system*
- Processed samples were prepared using DT and eDT workflows and analyzed using MBT Sepsityper® software extension
- Identification results were compared to MBT-CA Biotyper results from isolated colonies of strains used for contriving

Alverno Laboratories Fresh Sample Study

- In a companion study performed at Alverno Laboratories a total of 76 remnant patient PBC samples were tested
- Blood culture bottles were incubated in the BD BACTEC™ FX system with varying time post positivity for samples
- Remnant samples were processed locally at Alverno Laboratories using the Accelerate Arc BC kit and system*
- Processed samples were prepared using DT and eDT workflows and analyzed using MBT Sepsityper® software extension
- Identification results were compared to standard of care results from isolated colonies using MBT-CA Biotyper

STUDY RESULTS AND DISCUSSION

Accelerate Arc BC kit* and MBT Sepsityper® software extension on the MBT-CA system provide highly reportable and accurate ID results across a variety of sample types and species diversity (*Figure 4* & *5*)

- Data from both studies produced higher GN performance compared to GP performance
  - Total reportability (High+Low confidence scores) for GN samples was > 90% in both studies
  - Total reportability (High+Low confidence scores) for GP samples was lower at 80-83%
- Overall, reportability performance across all organisms combined was > 85% High or Low confidence ID
- In all cases, all reportable ID results from Accelerate Arc BC kit* processed samples matched the comparator ID result from testing of isolated colonies from culture using MBT-CA Biotyper
- For contrived samples, the number of strains tested per species was similar by design (~4-6 per species, ***Figure 6***)
- For fresh samples, the number of organisms tested and the distribution of strains per species was dependent on the prevalence for the site performing the study, Alverno Laboratories
- For fresh samples, more GP (45) than GN (31) organisms were tested with a majority of GP species identified being associated with common skin contaminants, including 20 from Coagulase negative *Staphylococci* (***Figure 6***)

Alverno Fresh Positive Blood Cultures

Organism Group	Reportability (High Confidence = ≥ 1.8 log(score); High+Low Confidence = ≥ 1.6 log(score))			Accuracy
	High	*High+ Low*	*No ID*	*Total*
GN (n=13 spp)	*83.9% (26/31)*	*93.5% (29/31)*	*6.5% (2/31)*	*100% (29/29)*
GP (n=24 spp)	*62.2% (28/45)*	*80.0% (36/45)*	*20.0% (9/45)*	*100% (36/36)*
All Orgs (n=37 spp)	*71.1% (54/76)*	*85.5% (65/76)*	*14.5% (11/76)*	*100% (65/65)*

Figure 5: ID Reportability and Accuracy Performance for fresh, remnant PBC samples
(Reportability per sample is defined by above criteria; each percentage shown includes the total number of samples tested per organism group, GN, GP, or All Orgs, i.e. combined; Accuracy is assessed by comparing only samples with a valid, reportable ID result to the comparator ID result from isolated colonies from culture of PBC)

CONCLUSIONS

- **The Accelerate Arc BC kit and system* can be used as a rapid and automated sample preparation method for positively-flagged BACTEC™ blood culture media for subsequent direct preparation of analyte spots for MBT Sepsityper® software extension analysis on the Bruker MBT-CA system**
- **The Accelerate Arc BC kit and system* can provide highly accurate ID results across a wide variety of bacterial species isolated from PBC samples**
- **Reportability performance from fresh PBC samples can be as high as 80% from GP organisms and > 90% from GN organisms**

ACKNOWLEDGEMENTS

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*** Pending 510(k) clearance. Not available for sale in the United States.**