Analytical Performance and Estimated Clinical Outcomes of a Molecular Multiplexed Bacterial Identification Blood Culture Panel

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Abstract

Introduction: Timely and accurate detection of bloodstream pathogens is critical in the diagnosis and treatment of patients with sepsis, where patients with bloodstream infections (BSI) have increased mortality, length of hospital stay and hospital costs. The ability of the clinical laboratory to provide a rapid identification of these organisms is vital to aide clinicians in empiric drug regimens and antibiotic management. Use of molecular multiplex panels, such as the GenMark Dx ePlex® Blood Culture Identification Gram Positive Panel (BCID-GP), that rapidly identify and provide some antimicrobial resistance data are becoming widely utilized in the clinical laboratory.

Methods: Positive blood cultures containing Gram-positive bacteria were run on the BCID-GP and compared to the standard of care (SOC). The BCID-GP analytical performance was determined for 71 blood cultures. To assess the potential impact to patient care, retrospective chart review was performed to estimate the number of antibiotic avoidances, de-escalation or escalation for patients included in the study.

Results: Median time savings for BCID-GP pathogen identification compared to SOC was 30 hours. Of the 71 samples tested, there were a total of 74 potential identifications and 26 resistance targets. There were three off-panel organisms encountered during this study, *Clostridium perfringens*, *Clostridium* species and *Finegoldia magna*, leaving 71 detectable targets. The percent agreement for identification was 95.7%, with 3 discrepant results. Two were poly-microbial BSIs where BCID-GP did not identify a Pan Gram Negative target when an *E. coli** and *P. aeruginosa* were present, but did identify *S. epidermidis* and *E. faecalis*, respectively. The other was a mono-microbial BSI with *S. salivarius* that was not detected. There was 100% agreement and 100% specificity for the genotypic resistance markers, *mecA* and *vanA*, compared to phenotypic SOC results. Retrospective chart review revealed 47.9% of patients had possible antibiotic stewardship interventions if using BCID-GP, with median time to faster intervention of 50.55 hours compared to SOC. Appropriate escalation to active therapy could have occurred sooner for Vancomycin-resistant enterococci and *Listeria*. Faster de-escalation, particularly for methicillin-sensitive *Staphylococcus aureus*, and complete avoidance of antibiotics would also be possible with BCID-GP results. Overall, 71 doses of vancomycin and 6 doses of linezolid could have been prevented, decreasing antibiotic costs and adverse side-effects of these drugs.

Conclusions: The BCID-GP is highly accurate in the identification of organisms and specific resistance genes from positive blood cultures, providing actionable results for antibiotic stewardship to administer more appropriate antibiotics and better patient care.

Abstract Revision Notice: Since the time of submission, one data error was recognized and additional data have been added. Overall conclusions have not changed. *Data error. *E. coli* was not detected in SOC.

Methods

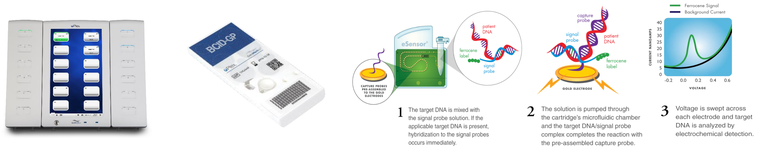
- First time positive blood cultures bottles identified by Gram-stain as having Gram-positive organisms (mono- or poly-microbial) were included in the study.
- SOC ID and AST was performed by MALDI-TOF-MS (Bruker) and Microscan (Beckman Coulter), respectively.
- Residual blood culture samples were run on GenMark Dx ePlex BCID-GP Research Use Only Panel, which detects the following targets:

Gram Positive Organisms
Bacillus cereus group
Bacillus subtilis group
Corynebacterium
Cutibacterium acnes
Enterococcus
Enterococcus faecalis
Enterococcus faecium
Lactobacillus
Listeria
Listeria monocytogenes
Micrococcus

Gram Positive Organisms
Staphylococcus
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus
Streptococcus agalactiae (GBS)
Streptococcus anginosus group
Streptococcus pneumoniae
Streptococcus pyogenes (GAS)

Resistance Genes
mecA
mecC
vanA
vanB
Pan Targets
Pan *Candida*
Pan Gram-Negative

The ePlex, BCID-GP Cartridge and the eSensor® Technology:



- Results from BCID-GP were compared to SOC for ID and AST, when appropriate. Estimated faster median time to ID was calculated compared to SOC.
- Retrospective chart review was performed to determine median time to antimicrobial stewardship (AMS) intervention compared to SOC, as well as potential opportunities intervention

Results

Table 1. Accuracy of BCID-GP ID compared to SOC. **(A)** Molecular ID from the BCID-GP was compared to the final MALDI-TOF-MS ID. Targets on BCID-GP that are genus or 'group' calls were considered correct if SOC gave same genus or species of that group. **(B)** Molecular genetic markers (*mecA* and *vanA*) were compared to phenotypic AST; *mecC* and *vanB* were not detected. GPC- Gram-positive cocci; GPR-Gram-positive rod; GNR-Gram-negative rods.

86 Patient Samples Tested for 91 potential IDs

3 samples containing off-panel organisms were removed from analysis (*Clostridium perfringens*, *Clostridium* species, *Finegoldia magna)*

88 on-panel targets analyzed

A

SOC Result by Gram stain Morphology		% Agreement ID	# ID Discrepant
GPC		98.8% (83/84)	1
GPR		100% (4/4)	0
Polymicrobial n=5	GP + 2nd GP n=2	100% (4/4)	0
	GP + GNR n=3	83% (5/6)	1
Overall		97.7% (86/88)	2

B

BCID-GP Genetic Resistance Call (n=69)	SOC Phenotypic AST	% Agreement
S. aureus *mecA* **not** detected	Methicillin susceptible	100% (21/21)
S. aureus *mecA* detected	Methicillin resistant	100% (10/10)
Coagulase-Negative Staphylococci *mecA* **not** detected	Oxacillin Susceptible	100% (7/7)
Coagulase-Negative Staphylococci *mecA* detected	Oxacillin Resistant	100% (14/14)
Enterococci *vanA* **not** detected	Vancomycin Sensitive	100% (11/11)
Enterococci *vanA* detected	Vancomycin Resistant	100% (6/6)

Table 2. Description of Polymicrobial Blood Cultures (n=5).

Gram stain morphology	SOC ID	BCID-GP ID	Organism Missed by BCID-GP	BCID-GP Target ID not on Gram-stain
GPC pairs, chains and clusters	*S. aureus* *S. constellatus*	*S. aureus* *S. anginosus* group	None	N/A
GPC	*S. mitis* group *P. aeruginosa*	*Streptococcus* Pan-GN	None	Pan-GN
GPC pairs and chains	*E. faecalis* *P. aeruginosa*	*E. faecalis*	*P. aeruginosa* (No Pan-GN call detected)	N/A
GPC pairs and chains	*E. faecalis* *K. pneumoniae*	*E. faecalis* Pan-GN	None	Pan-GN
GPC pairs and chains	*E. faecalis* *Staphylococcus*	*E. faecalis* *S. hominis*	None	*Staphylococcus*

Table 3. Discrepant ID Results. ePlex data files were analyzed by GenMark Diagnostics for target missed to confirm lack of signal. Samples were not re-tested due to limited kits.

Morphology seen on Gram stain	SOC Culture and ID	BCID-GP Panel ID
GPCpc	*S. salivarius*	No target detected
GPCpc	*E. faecalis* + *P. aeruginosa*	*E. faecalis*

Results

Figure 1. Estimated Time Savings using BCID-GP. **(A)** Anticipated time to BCID-GP result was calculated by adding 2 hours to the time of bottle positivity to account for Gram-stain preparation and BCID-GP run time (90 minutes). Theoretical difference in time to ID using BCID-GP compared to SOC was determined by subtracting the BCID-GP time from the time to ID by SOC. **(B)** Estimated time to stewardship intervention was calculated based on the time the BCID-GP results would be available. Theoretical difference in time to intervention was calculated by subtracting BCID-GP intervention time from intervention made based on SOC results.

A Median Faster Time to ID

B Median Faster Time to AMS Intervention

Figure 2. Anticipated Stewardship Interventions based on BCID-GP Results.
Retrospective chart review was performed to determine stewardship interventions that could be made for those patients based on BCID-GP result.

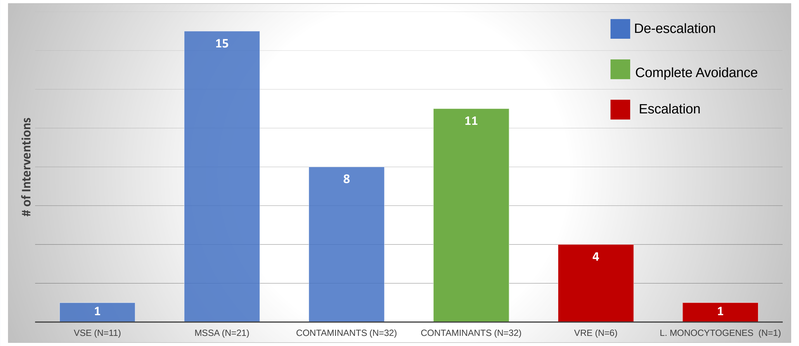


Table 4. Estimated Stewardship Interventions by Pathogen

Organism	Phenotype	Intervention Type						
		Escalation	De-escalation	Avoid Vancomycin	# doses saved	Avoid Linezolid	# doses saved	Complete Avoidance of Antibiotics
Enterococci	Vancomycin Susceptible n=11	0	1	1	3	1	6	-
	Vancomycin Resistant n=6	3	0	0	0	-	-	-
S. aureus	Methicillin Sensitive n=21	0	15	4	44	-	-	-
	Methicillin Resistant n=10	0	0	0	0	-	-	-
Streptococci	*Streptococci* spp.* n=9	0	3	0	12	-	-	11
Potential Contaminants	*S. epidermidis* n=15	0	4	2	17	-	-	
	Other CoNS n=6	0	0	2	3	-	-	
	Other n=4	0	1	1	2	-	-	
Other species	*Listeria monocytogenes* n=1	1	0	1	0	-	-	-

* Includes one *S. pneumoniae* and one *S. anginosus* group

Conclusions

- GenMark Dx BCID-GP was highly accurate in bacterial ID during our validation study.
  - Overall accuracy= **97.7%**
- BCID-GP resistance calls, based on presence or absence of genetic resistance markers *mecA* and *vanA*, agreed **100%** with phenotypic testing.
  - No major or very major errors occurred.
- BCID allowed for faster time to ID and stewardship interventions, with median time to a faster ID of **29.3** hours and median time to a faster intervention of **50.55** hours.
- We estimate that stewardship interventions were possible in **50%** of patients based on BCID-GP results:
  - Escalation in 4 cases
  - De-escalation in 24 cases
  - Complete avoidance of antibiotics in 11 cases
- Implementation of BCID-GP may aid in faster diagnosis and treatment of patients with GP BSI.