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Clinical Performance of the Novel GenMark Dx ePlex® Blood Culture Identification Gram Positive Panel

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ABSTRACT

Background: Rapid diagnosis of positive blood cultures is standard of care (SOC) in many clinical laboratories. The GenMark ePlex Blood Culture ID Gram Positive (BCID-GP) Panel is a novel multiplex assay based on competitive DNA hybridization and electrochemical detection using proprietary eSensor® technology. This multicenter study compared the BCID-GP Panel to comparative methods for identification of 20 gram-positive bacteria, 4 resistance genes, Pan *Candida* and Pan Gram-Negative assays unique to the BCID-GP Panel. **Materials and Methods**: Ten clinical laboratories throughout the US collected remnant, de-identified positive blood culture samples for analysis. Five laboratories tested clinical and contrived samples with the BCID-GP Panel. Comparative identification methods were the laboratory's SOC, including MALDI-TOF MS and automated identification systems, and targeted PCR/qPCR with bidirectional sequencing. Sensitivity and specificity were determined for each target. **Results:** A total of 2,354 samples (1,788 clinical, 566 contrived) were tested with the BCID-GP Panel. For pathogenic gram-positive targets (*B. cereus* group, *Enterococcus*, *E. faecalis*, *E. faecium*, *Staphylococcus*, *S. aureus*, *S. lugdunensis*, CoNS, *Listeria*, *L. monocytogenes*, *Streptococcus*, *S. agalactiae*, *S. anginosus* group, *S. pneumoniae*, *S. pyogenes*), sensitivity and specificity ranged from 93.2%-100% and 99.0%-100%, respectively. For contamination rule-out targets (*B. subtilis* group, *Corynebacterium*, *C. acnes*, *Lactobacillus*, *Micrococcus*), sensitivity and specificity ranged from 84.5%-100% and 99.9%-100%, respectively. Sensitivity and specificity for the Pan *Candida* and Pan Gram-Negative targets were 92.4%, 95.7% and 99.9%, 99.6%, respectively. The sensitivity for each resistance marker was *mecA* 97.2%, *mecC* 100%, *vanA* 96.8%, and *vanB* 100%. Specificity ranged from 96.7%-100%. **Conclusion:** The ePlex BCID-GP Panel compares favorably to SOC and targeted molecular methods. This Panel detects a broad range of pathogens and, unlike other tests, mixed infections with yeast and gram-negative organisms in the same positive blood culture bottle.

INTRODUCTION

- Sepsis ranks among the top 10 causes of death and the incidence of bacteremia and candidemia seems to be increasing.
- Rapid diagnosis of positive blood cultures, especially when combined with antimicrobial stewardship, has been shown to reduce the interval between recognition of a bacteremia and appropriate therapy by 18-24 h or longer[1].
- Routinely used nucleic acid methods are limited by the number of pathogens they can detect and almost all have difficulty with differentiation of polymicrobial bacteremia.
- The GenMark Dx ePlex® Gram-Positive Blood Culture Identification Panel (BCID-GP) is an investigational qualitative nucleic acid multiplex assay intended for use on the ePlex that detects 20 gram positive species or groups and *mecA*, *mecC*, *vanA*, and *vanB* resistance genes.
- The panel also contains Pan *Candida* and Pan Gram-Negative probes.

METHODS

A total of 2,354 samples (1,788 clinical, 566 contrived) were tested with the BCID-GP Panel.

- Remnant positive blood cultures from subjects of all ages and genders
- 10 geographically diverse US sites, 5 testing sites

Comparator methods for organism identification:

- Traditional culture
- FDA-cleared MALDI-TOF MS (i.e., bioMérieux® Vitek® MS, Bruker® Biotyper®)
- Automated microbiological and biochemical techniques (e.g., Becton Dickinson® [BD] Phoenix™, bioMérieux® Vitek® 2, Siemens® MicroScan®)
- Samples with *Corynebacterium*, *S. epidermidis*, *S. hominis*, or *C. parapsilosis* identified by SOC were confirmed using analytically validated PCR amplification assays followed by bi-directional sequencing (PCR/sequencing) or 16S rRNA gene sequencing (2-4). The comparator methods for ARGs were validated quantitative PCR (qPCR) amplification assay(s) followed by bi-directional sequencing for samples with the associated organisms identified (i.e., *Staphylococcus, Enterococcus*).

Discordant Resolution Method

- Discordant results between the BCID-GP Panel and the comparator method(s) were tested with PCR/sequencing to determine the presence or absence of the organism.

Statistical Methods

- PPA was calculated as 100 x #TP/ (#TP+#FN); NPA was calculated as 100 x #TN/ (#TN+#FP) where TP=true positive, FN=false negative, TN=true negative, and FP=false positive. The two-sided 95% score confidence interval (CI) was calculated for PPA and NPA.

RESULTS

Data Post Discordant Resolution

ePlex BCID-GP Target	Overall Positive Percent Agreement with Discordant Resolution		Overall Negative Percent Agreement with Discordant Resolution	
	TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
***Bacillus cereus* group**	57/58	98.3 (90.9-99.7)	1804/1804	100 (99.8-100)
***Bacillus subtilis* group**	52/52	100 (93.1-100)	1809/1809	100 (99.8-100)
Corynebacterium	62/68	91.2 (82.1-95.9)	1794/1794	100 (99.8-100)
Cutibacterium acnes	44/47	93.6 (82.8-97.8)	1814/1815	99.9 (99.7-100)
Enterococcus	327/334	97.9 (95.7-99.0)	1528/1528	100 (99.7-100)
Enterococcus faecalis	183/187	97.9 (94.6-99.2)	1674/1674	100 (99.8-100)
Enterococcus faecium	128/130	98.5 (94.6-99.6)	1728/1731	99.8 (99.5-99.9)
Lactobacillus	46/47	97.9 (88.9-99.6)	1814/1815	99.9 (99.7-100)
Listeria	76/77	98.7 (93.0-99.8)	1784/1785	99.9 (99.7-100)
Listeria monocytogenes	48/48	100 (92.6-100)	1814/1814	100 (99.8-100)
Micrococcus	66/68	97.1 (89.9-99.2)	1793/1794	99.9 (99.7-100)
Staphylococcus	746/756	98.7 (97.6-99.3)	1105/1106	99.9 (99.5-100)
Staphylococcus aureus	346/352	98.3 (96.3-99.2)	1429/1430	99.9 (99.6-100)
Staphylococcus epidermidis	152/156	97.4 (93.6-99.0)	1591/1607	99.0 (98.4-99.4)
Staphylococcus lugdunensis	53/53	100 (93.2-100)	1709/1710	99.9 (99.7-100)
Streptococcus	339/344	98.5 (96.6-99.4)	1517/1518	99.9 (99.6-100)
Streptococcus agalactiae	55/55	100 (93.5-100)	1779/1780	99.9 (99.7-100)
***Streptococcus anginosus* group**	66/66	100 (94.5-100)	1767/1769	99.9 (99.6-100)
Streptococcus pneumoniae	67/67	100 (94.6-100)	1766/1767	99.9 (99.7-100)
Streptococcus pyogenes	53/54	98.1 (90.2-99.7)	1780/1780	100 (99.8-100)
Pan *Candida*	98/105	93.3 (86.9-96.7)	2236/2237	100 (99.7-100)
Pan Gram-Negative	424/442	95.9 (93.7-97.4)	1895/1900	99.7 (99.4-99.9)
mecA	416/426	97.7 (95.7-98.7)	319/326	97.9 (95.6-99.0)
mecC	49/49	100 (92.7-100)	703/703	100 (99.5-100)
vanA	122/124	98.4 (94.3-99.6)	209/210	99.5 (97.4-99.9)
vanB	53/53	100 (93.2-100)	281/281	100 (98.7-100)

RESULTS

Pre discordant resolution, for pathogenic gram-positive targets, sensitivity and specificity ranged from 93.2%-100% and 99.0%-100%, respectively. Post discrepant analysis results are displayed in the **Table.** For contamination rule-out targets (*B. subtilis* group, *Corynebacterium*, *C. acnes*, *Lactobacillus*, *Micrococcus*), sensitivity and specificity ranged from 84.5%-100% and 99.9%-100%, respectively. The sensitivity for each resistance marker was *mecA* 97.2 %, *mecC* 100%, *vanA* 96.8%, and *vanB* 100%. Specificity ranged from 96.7%-100%.

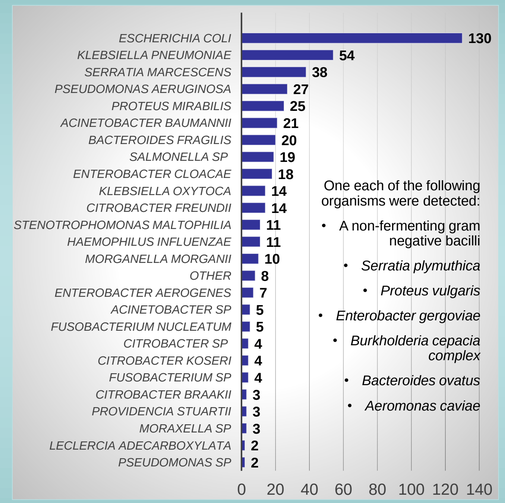

Fig 1: Pan Gram-Negative targets
Overall sensitivity, specificity 95.9%, 99.7%

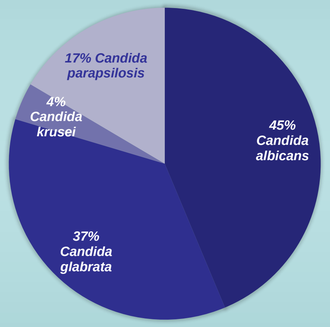

Fig 2: Pan *Candida* targets
Overall sensitivity, specificity 93.3%, 100%

CONCLUSIONS

The ePlex BCID-GP Panel compares favorably to SOC and targeted molecular methods. Compared to other commercially available platforms, this assay detects a broader range of pathogens. Unlike other tests, ePlex BCID-GP Panel detects mixed infections with yeast and gram-negative organisms in the same positive blood culture bottle. The contamination targets have the potential to reduce inappropriate antibiotic utilization.

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