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Preliminary Evaluation of the Research-Use-Only (RUO) iCubate iC-GPC Assay for Identification of Select Gram-Positive Bacteria and Their Resistance Determinants in Blood Culture Broths

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The iC-GPC assay (iCubate, Huntsville, AL) provides a molecular option for the rapid, on-demand analysis of positive blood cultures. A preliminary evaluation of the iC-GPC assay using 203 clinical or seeded specimens demonstrated a sensitivity of 93.8% to 100% and a specificity of 98.0% to 100% for the identification of five Gram-positive bacterial species (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium*) and three associated genetic resistance determinants (*mecA*, *vanA*, and *vanB*) in positive blood culture broths.

The rapid identification of bacterial and fungal pathogens in positive blood culture broths by use of a variety of methods has been described. These methods include peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH), matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), and real-time PCR (RT-PCR) or microarray-based molecular tests (1–10). The ability to reliably identify a specific bacterium or yeast present in a positive blood culture within 1 to 3 h of culture positivity using these methods has resulted in significant reductions in time to effective antimicrobial therapy, length of hospital/intensive care unit (ICU) stay, 30-day mortality, and cost of care (6, 7, 11–13). Importantly, while organism identification alone can provide some benefit, the most significant benefits are achieved when the presence of resistance markers, such as *mecA*, *vanA*, or carbapenemases, is identified concomitantly (11, 12, 14–16).

The research-use-only (RUO) iC-GPC assay (iCubate, Huntsville, AL) is a molecular target amplification assay capable of detecting and identifying *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium* as well as the genetic resistance determinants *mecA*, *vanA*, and *vanB* directly from positive blood culture broths. The system consists of an automated processor (iC-Processor), a reader (iC-Reader), and single-use, closed-system test cassettes. Each test cassette contains all reagents necessary for cell lysis, nucleic acid extraction, target amplification, and amplicon hybridization to an array of immobilized capture probes. Each immobilized capture probe has a unique nucleic acid sequence, which can hybridize to the target. A second fluorescence-labeled gene-specific detection probe contained within the closed cassette was used to detect the target after capture.

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We conducted a preliminary evaluation of the iC-GPC assay using a total of 215 positive blood culture broths containing Gram-positive cocci (GPC). Positive broths were enrolled and tested at three clinical laboratories. The cohort included 107 prospectively collected blood cultures and was augmented with 108 simulated blood cultures seeded with organisms less frequently encountered in prospective specimens (35 cultures containing *E. faecalis*, 49 containing *E. faecium*, and 24 containing *S. pneumoniae*). For inclusion in the study, blood cultures had to contain GPC upon primary Gram stain and be available for iC-GPC testing within 24 h of culture positivity ($n = 41$) or frozen at <-70°C for analysis at a later time ($n = 57$). Cultures containing Gram-negative organisms in addition to GPC (i.e., mixed cultures) were not enrolled. Simulated seeded cultures were constructed using unique (i.e., nonredundant) isolates originally obtained from clinical specimens at each test site. A fresh subculture of each isolate was used to make a suspension at 10^2 to 10^3 CFU/ml in 0.65% NaCl. A 1.0-ml portion of this suspension was used to inoculate a residual blood culture broth, which was negative after 5 days of incubation. Inoculated blood culture bottles were then reinserted into an automated blood culture incubator until they signaled positive. A 10-μl portion of the positive broth (prospective or simulated) was transferred to the sample well within the iC cassette, which was then inserted into the iC-Processor for automated specimen processing and array hybridization (~4.5 h). Following processing, the iC cassette was transferred to the iC-Reader for analysis (~5 min).

Results obtained from the iC-GPC assay were compared to those obtained from the culture-based standard-of-care method used at each clinical test site. This included subculture of the positive broth to solid medium and identification of the resulting colonies by use of MALDI-TOF MS or routine biochemical tests. The identity of any isolate identified as *S. pneumoniae* by MALDI-

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TABLE 1 Performance of iC-GPC for identification of select Gram-positive cocci and their associated resistance determinants in positive blood culture broths[a]

Target	No. of cultures					Sensitivity (%) (CI)	Specificity (%) (CI)
	TP	TN	FP	FN	Total		
Bacterial identification							
S. aureus	24	177	2[b]	0	203	100.0 (83–100)	98.9 (96–99)
S. epidermidis	39	162	1[c]	1[d]	203	97.5 (85–99)	99.4 (96–100)
S. pneumoniae	30	171	0	2[e]	203	93.8 (78–99)	100.0 (97–100)
E. faecalis	40	162	0	1	203	97.6 (86–99)	100.0 (97–100)
E. faecium	47	154	2[f]	0	203	100.0 (91–100)	98.7 (95–99)
Resistance determinant(s)							
mecA[g]	17	15	0	0	32	100.0 (77–100)	100.0 (74–100)
vanA, *vanB*[h]	35	49	1[i]	2[j]	87	94.6 (80–99)	98.0 (88–99)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative; CI, 95% confidence interval.
[b] The broths contained *E. faecalis* (1 culture) and *S. lentus* (1 culture) by reference culture.
[c] The broths contained both *S. pyogenes* and *S. agalactiae* by reference culture.
[d] Reported as *S. aureus* by iC-GPC. The broth contained both *S. aureus* and *S. epidermidis* by reference culture.
[e] Reported as "not detected" for all targets by iC-GPC.
[f] Reported as positive for both *S. epidermidis* and *E. faecalis* (1 culture) or both *S. pneumoniae* and *E. faecalis* (1 culture) by iC-GPC. *E. faecalis* was not isolated from either broth by reference culture.
[g] Susceptibility results for cefoxitin were available for 32/63 isolates identified as either *S. aureus* or *S. epidermidis* by iC-GPC and culture. Results were used to infer the presence or absence of *mecA*.
[h] Susceptibility results for vancomycin were available for all isolates identified as *E. faecalis* or *E. faecium*. Results were used to infer the presence or absence of *vanA* or *vanB*. *vanA* and *vanB* are separate targets on the iC-GPC panel but were not differentiated by vancomycin MIC result.
[i] The broth contained *E. faecalis*, which tested as susceptible to vancomycin.
[j] The broths contained *E. faecalis*, which tested as resistant to vancomycin.

TOF MS was confirmed using an optochin disk diffusion test. Antimicrobial susceptibility tests were performed for isolates identified as *S. aureus* or *S. epidermidis* (cefoxitin) and *E. faecalis* or *E. faecium* (vancomycin) to infer the presence of *mecA* resistance markers or *vanA* and *vanB* resistance markers, respectively.

The iC-GPC assay generated an initial invalid result for 23/215 (10.7%) broths tested. The most common source of invalid test results was a "positive control check fail" error (meaning that the internal process control was not detected), which may indicate the presence of inhibitory substances in these samples. Six specimens could not be reanalyzed within the 24-h time period following culture positivity indicated by the study protocol. Seventeen specimens were reanalyzed in a second iC-GPC assay, and 11/17 (64.7%) generated a valid result.

In all, 203 blood cultures with valid iC-GPC results were used to establish the performance characteristics of the iC-GPC assay for the identification of five bacterial species and three genetic resistance markers (Table 1). Cultures with false-negative results for *S. epidermidis* ($n = 1$) and *S. pneumoniae* ($n = 2$) were reported as "not detected" in the iC-GPC assay. The limit of detection (LoD) for iC-GPC targets is approximately 10^6 CFU/ml (established by the manufacturer), which is below the concentration of 10^7 to 10^8 CFU/ml typically required for a broth culture to signal as positive in automated blood culture systems (17). It is possible that these cultures signaled early and contained bacterial concentrations below the assay LoD; however, bacterial growth in positive broth cultures was not quantitated in this study. The culture with a false-negative result for *E. faecalis* was reported as positive for *S. aureus* and *mecA* by iC-GPC. This was a simulated culture which was seeded with *E. faecalis*. This culture also accounts for one of two false-positive results observed for *S. aureus*. It is difficult to explain these results since there are no *in silico* homologous regions between the primer and probe sequences and the targeted sequences used to identify *S. aureus* and *E. faecalis*. Possible explanations are a specimen mislabel or mix-up during testing. However, we were unable to confirm either of these possible errors. The remaining false-positive result for *S. aureus* was observed for a prospective broth culture from which *S. lentus* was isolated and identified by the reference culture method. The single false-positive result for *S. epidermidis* was observed for a culture that was positive for both *Streptococcus agalactiae* and *Streptococcus pyogenes* by the reference culture method. A possible explanation is that this false-positive result represents a low level of *S. epidermidis* in the blood culture broth which was not recovered by conventional culture; however, currently there is no evidence to support this explanation. Two additional cultures with false-positive results were both reported as *E. faecium* by iC-GPC. One of the two cultures was a simulated specimen that was seeded with *S. pneumoniae* but was reported as positive for both *S. pneumoniae* and *E. faecalis* by iC-GPC; the other was a prospective culture that contained only *S. epidermidis* by the reference culture but was positive for both *S. epidermidis* and *E. faecalis* by iC-GPC. Again, in both instances it is possible that this represents a small amount of *E. faecalis* present in the blood culture broth which was not recovered by conventional culture.

The iC-GPC assay relies on amplification of the target prior to detection, which may result in additional positive results for organisms present in blood cultures that fail to grow due to the presence of antibiotics or because the organisms were present as a minority population in the specimen. Alternatively, this could represent free nucleic acid present in the specimen or amplicon contamination. Importantly, Gram stain of the primary specimen in each case revealed only a single bacterial morphology. Eighteen positive blood broths contained GPC that were not identified by the iC-GPC assay. This included 12 cultures with various coagulase-negative *Staphylococcus* spp. (CoNS) and 6 containing vari-

ous *Streptococcus* spp. (2 containing *S. agalactiae*, 2 containing the *Streptococcus mitis/Streptococcus oralis* group, 1 containing *Streptococcus anginosus*, and 1 containing viridans group streptococci).

Results of cefoxitin susceptibility testing were available for 32/63 (50.8%) of isolates identified as *S. aureus* ($n = 20$) or *S. epidermidis* ($n = 12$) by the iC-GPC assay and culture. The remaining cultures containing *Staphylococcus* spp. were tested using a method other than cefoxitin disk diffusion (*S. aureus*) ($n = 4$) or were deemed not to be clinically significant (*S. epidermidis*) ($n = 27$) and were excluded from analysis. All isolates reported as positive for *mecA* by iC-GPC also tested as resistant to cefoxitin, whereas all isolates reported as negative for *mecA* were susceptible. Results for vancomycin susceptibility were available for all 87 isolates identified by iC-GPC and culture as *E. faecalis* ($n = 40$) or *E. faecium* ($n = 47$). Both false-negative results were observed for simulated cultures seeded with *E. faecalis* which tested as resistant to vancomycin at an MIC of >256 μg/ml, which suggests the presence of *vanA*.

This preliminary evaluation of the iC-GPC assay demonstrated high sensitivity (≥93.8%) and specificity (≥98.7%) for the identification of each of five bacterial species targets and two genetic determinants of resistance (vancomycin resistances due to *vanA* and *vanB* were not differentiated in this study). The panel of 5 bacterial identification targets present in the iC-GPC assay is not as comprehensive as the panels for other multiplexed molecular tests: the Verigene BC-GP assay (Nanosphere, Northbrook, IL) detects 12 organisms, and the FilmArray BC-ID assay (bioMérieux, Durham, NC) detects 19 bacterial targets; however, the iC-GPC assay still identified the organism present in 83/101 (82.1%) prospectively collected blood cultures with an initial Gram stain of Gram-positive cocci. The majority (15/18, 83.3%) of cultures containing organisms not on the iC-GPC panel were various CoNS or viridans group streptococci.

A weakness of the study was the inclusion of a large proportion of simulated cultures to establish the performance of the iC-GPC assay. This was necessary to fully evaluate all targets on the panel, including some organisms not commonly found in positive blood cultures. Clinical blood cultures that were negative at 5 days of incubation were used as a matrix, and unique clinical isolates were used to seed the cultures prior to incubation in an automated blood culture system to create simulated cultures that closely mimic real clinical samples. A potential shortcoming of this method would be the presence of a small amount of organism (viable or not) in the "negative" clinical cultures used as a matrix. The presence of these "residual" organisms in the matrix could potentially contribute to the false-positive results observed for simulated cultures.

A potential benefit of the iC-GPC assay is the use of a single, closed-system consumable cassette. This enables a simplified assay setup (<5 min of hands-on time) and also aids in reducing the risk of aerosolization of potentially infectious organisms and amplicon contamination. Furthermore, the iC-Processor is capable of random-access processing of up to four test cassettes simultaneously by use of an instrument that has a relatively small footprint (17 by 17 by 16 in.). Combined, these attributes may positively impact safety, workflow, and throughput for the direct identification of bacteria present in positive blood cultures.

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