Parallel Evaluation of the MALDI Sepsityper and Verigene BC-GN Assays for Rapid Identification of Gram-Negative Bacilli from Positive Blood Cultures

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ABSTRACT Rapid identification of microorganisms from positive blood cultures has improved clinical management and antimicrobial stewardship. The advent of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has reduced the time to identification of cultured isolates and is now often the definitive method used in the clinical microbiology laboratory. The commercial *in vitro* diagnostic MALDI Sepsityper (Sepsityper) kit has the potential for standardization and clinical routine use for the rapid identification of a broad range of bacteria from positive blood cultures. In this study, we performed a parallel evaluation of the Sepsityper (Bruker Daltonics, Billerica, MA) and the Verigene BC-GN (BC-GN) assays (Nanosphere, Inc., Northfield, IL) for the identification of Gram-negative bacilli. A total of 210 Bactec bottles demonstrating Gram-negative bacilli were prospectively enrolled for this study. Among these, 200 monomicrobial cultures were included in the comparative analysis. For monomicrobial cultures, the BC-GN detected 85% (170/200) compared to that detected by routine culture while the Sepsityper detected 94% (188/200) and 91% (181/200) to the genus and species levels, respectively. Comparable positive percentage agreement and negative percentage agreement were observed between the Sepsityper (96.5% and 98.8%, respectively) and the BC-GN (99.4% and 99.8%, respectively) when only ($n = 170$, 85%) organisms targeted by the latter test were included in the analysis. In conclusion, the two methods evaluated in this study showed excellent performance characteristics for the identification of Gram-negative bacilli commonly isolated from blood cultures. The Sepsityper showed a broader identification range capability that may further improve clinical management and antimicrobial stewardship in patients with less frequent Gram-negative bacilli bloodstream infections.

KEYWORDS Gram-negative bacilli, MALDI-TOF, Verigene BC-GN, blood culture

Bloodstream infections (BSIs) are an important cause of morbidity and mortality (1). Following the onset of sepsis-related hypotension, mortality rate increases nearly 8% for every hour that effective antimicrobial therapy is delayed (2). Therefore, rapid identification (ID) of the causative agent and antimicrobial susceptibility testing (AST) are important to improve patient outcome. Routine microbiology methods rely on subculture of positive blood cultures to solid medium and require 18 to 48 h, or more, from initial blood culture positivity to definitive bacterial ID and AST. To address this challenge, several diagnostic systems have been developed and marketed for rapid ID of organisms found in positive blood cultures (3, 4).

These new emerging technologies offer the advantage of same-day ID of potential

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bacterial pathogens and detection of antibiotic-resistant markers directly from blood cultures. Several studies have demonstrated that the addition of such rapid ID methods improves clinical management and antimicrobial stewardship by shortening the time to effective therapy, leading to better use of antimicrobials, decreasing risk of antimicrobial resistance, minimizing adverse effects, shortening hospital stays, and lowering overall health care cost (4–9). Despite these benefits, complex sample handling, insufficient species coverage, and high assay cost have hampered widespread usage of these methods in routine blood culture processing (4).

Application of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has reduced the time to ID of cultured isolates and is now considered the definitive ID method in the clinical microbiology laboratory (10). The commercial *in vitro* diagnostic Bruker's MALDI Sepsityper kit (Sepsityper) has the potential for clinical routine use and standardization since the manufacturer is responsible for its regulatory approval and the quality of the product. Use of this technology has been extended to identify bacteria directly from positive blood cultures with some success (11–13). A review article written by Morgenthaler and Kostrzewa in 2015 summarizes the performance of the Sepsityper from a meta-analysis of 21 published reports (11). As described by the authors, many of these studies differ in applied procedures, the gold standards with which the Sepsityper was compared, MALDI-TOF MS log(score) cutoff values used for pathogen ID, and distribution of the bacteria isolated from bacteremic patients. Their study results showed that the Sepsityper allowed reliable ID at the species level in 80% of 3,320 blood culture bottles. Specifically, consistently higher ID rates for Gram-negative bacteria (90%) were achieved compared to those for Gram-positive bacteria (76%) or yeast (66%). Based on these results and despite the study limitations, the authors concluded that the Sepsityper was equal or superior to other extraction procedures.

The Sepsityper is currently a research use only kit that in combination with the MALDI Biotyper enables the rapid identification of Gram-positive bacteria, Gram-negative bacteria, and yeast. The Sepsityper rapid blood culture-to-ID workflow requires ~30 min of hands-on time from processing to identification, depending on the number of samples being processed. Notably, the average materials cost is ~\$5 to \$9 per test. In contrast, the Verigene blood culture Gram-negative test (BC-GN) is an FDA-approved, automated microarray-based, multiplexed nucleic acid test for the rapid identification of the most common Gram-negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., and *Acinetobacter* spp.) and detection of their drug resistance markers (KPC, NDM, CTX-M, VIM, IMP, and OXA). The BC-GN is a sample-to-result test that requires 5 min of hands-on time and ~2.5 h of run time per sample. However, the materials cost is substantially higher (~\$60 to \$80 per test) compared to the Sepsityper. All things considered, the Sepsityper affordability and broader detection capability for the rapid ID of organisms found in positive blood cultures make this test an attractive alternative compared to other tests like the Verigene BC-GN.

In this study, we present the results of a parallel evaluation of the Sepsityper using the MALDI Biotyper (MBT) Sepsityper software module against the BC-GN assays for the ID of Gram-negative bacilli. Identification results of the two assays were compared with those obtained by MALDI-TOF MS for bacteria isolated on solid media. To our knowledge, this is the first parallel evaluation between these two assays.

RESULTS

Distribution of bacterial isolates from positive blood cultures. The distribution of microorganisms isolated from 210 positive blood cultures is shown in Table 1. Overall, the most common isolated Gram-negative bacilli were *E. coli* ($n = 83$, 37.9%), *K. pneumoniae* ($n = 33$, 15.1%), *P. aeruginosa* ($n = 21$, 9.6%), *Proteus mirabilis* ($n = 11$, 5%), and *Enterobacter cloacae* complex ($n = 10$, 4.6%). Among the 210 positive blood cultures with Gram-negative bacilli, 200 were monomicrobial and 10 were misinterpreted by Gram stain and/or polymicrobial. A total of 157 (78.5%) of the monomicrobial

TABLE 1 Distribution of microorganisms isolated from 210 positive blood cultures

Definitive identification	No. of monomicrobial isolates	No. of polymicrobial isolates	Total no. (%) of isolates
<i>Enterobacteriaceae</i>	157	6	163 (74.4)
<i>Escherichia coli</i>	82	1	83 (37.9)
<i>Klebsiella pneumoniae</i>	31	2	33 (15.1)
<i>Proteus mirabilis</i>	10	1	11 (5.0)
<i>Enterobacter cloacae</i> complex	10	0	10 (4.6)
<i>Enterobacter aerogenes</i>	6	0	6 (2.7)
<i>Serratia marcescens</i>	6	0	6 (2.7)
<i>Klebsiella variicola</i>	4	0	4 (1.8)
<i>Klebsiella oxytoca</i>	3	0	3 (1.4)
<i>Morganella morganii</i>	2	0	2 (0.9)
<i>Citrobacter freundii</i> complex	1	0	1 (0.5)
<i>Enterobacter amnigenus</i>	1	0	1 (0.5)
<i>Providencia rettgeri</i>	1	0	1 (0.5)
<i>Providencia stuartii</i>	0	1	1 (0.5)
<i>Kluyvera</i> spp.	0	1	1 (0.5)
<i>Non-Enterobacteriaceae</i>	43	4	47 (21.5)
<i>Pseudomonas aeruginosa</i>	21	0	21 (9.6)
<i>Acinetobacter baumannii</i> complex	3	0	3 (1.4)
<i>Achromobacter xylosoxidans</i>	2	0	2 (0.9)
<i>Stenotrophomonas maltophilia</i>	2	0	2 (0.9)
<i>Bacteroides fragilis</i>	2	0	2 (0.9)
<i>Fusobacterium</i> spp.	2	0	2 (0.9)
<i>Haemophilus influenzae</i>	2	0	2 (0.9)
<i>Acinetobacter lwoffii</i>	1	1	2 (0.9)
<i>Haemophilus paraphrohaemolyticus</i>	0	1	1 (0.5)
<i>Acinetobacter junii</i>	1	0	1 (0.5)
<i>Aeromonas caviae</i>	1	0	1 (0.5)
<i>Alistipes fingoldii</i>	1	0	1 (0.5)
<i>Bacteroides thetaiotaomicron</i>	1	0	1 (0.5)
<i>Moraxella osloensis</i>	1	0	1 (0.5)
<i>Neisseria flavescens</i>	0	1	1 (0.5)
<i>Prevotella denticola</i>	1	0	1 (0.5)
<i>Prevotella buccae</i>	1	0	1 (0.5)
<i>Rhizobium radiobacter</i>	1	0	1 (0.5)
<i>Veillonella</i> spp.	0	1	1 (0.5)
Gram-positive organisms ^a	4	5	9 (4.1)
Total	204	15	219

^aReported as Gram-negative bacilli on blood culture Gram stain report (see Tables 2).

isolates belonged to the *Enterobacteriaceae* family. The remaining 43 (21.5%) isolates were grouped as non-*Enterobacteriaceae*. Within this group, only 10 (5%) were anaerobic Gram-negative bacilli. The distribution of positive blood culture bottle types used in this study was as follows: Bactec Lytic/10 Anaerobic/F ($n = 115$), Bactec Plus Aerobic/F ($n = 88$), and Bactec Peds Plus/F medium ($n = 7$). The blood bottle types used in this study did not appear to influence the performance of the methods evaluated (data not shown).

Identification of Gram-negative bacilli. The performances of the BC-GN and Sepsityper assays for the ID of Gram-negative bacilli from positive monomicrobial blood cultures are summarized in Table 2. Overall, the BC-GN correctly identified 85% (170/200) of the organisms isolated while the Sepsityper correctly identified 91% (182/200) and 94% (188/200) of the organisms isolated at the species and genus levels, respectively. The Sepsityper demonstrated improved performance for the ID of organisms within the *Enterobacteriaceae* group compared to that for the non-*Enterobacteriaceae* group. At the genus level, the Sepsityper showed 98.1% (154/157) concordance for *Enterobacteriaceae* compared to 79.1% (34/43) for non-*Enterobacteriaceae* isolates ($P < 0.001$). At the species level, the Sepsityper showed

TABLE 2 Evaluation of Verigene BC-GN and MALDI Sepsityper for 200 monomicrobial blood cultures

Definitive identification	No. (%) of cultures	No. (%) organisms correctly detected by:		
		MALDI Sepsityper		Verigene BC-GN
		Genus (>1.6)	Species ($\geq$ 1.8)	
<i>Enterobacteriaceae</i>	157 (78.5)	154 (98.1)	149 (94.9)	144 (91)
<i>Escherichia coli</i>	82 (41)	82 (100)	80 (97.6)	82 (100)
<i>Klebsiella pneumoniae</i>	31 (15.5)	30 (96.8)	30 (96.8)	28 (90.3)
<i>Enterobacter cloacae</i> complex	10 (5)	9 (90)	9 (90)	10 (100)
<i>Proteus mirabilis</i>	10 (5)	9 (90)	9 (90)	10 (100)
<i>Enterobacter aerogenes</i>	6 (3)	6 (100)	6 (100)	6 (100)
<i>Serratia marcescens</i>	6 (3)	6 (100)	6 (100)	NI ^a (100)
<i>Klebsiella variicola</i>	4 (2)	4 (100)	2 (50)	3 (75) ^b
<i>Klebsiella oxytoca</i>	3 (1.5)	3 (100)	3 (100)	3 (100)
<i>Morganella morganii</i>	2 (1)	2 (100)	2 (100)	NI (100)
<i>Citrobacter freundii</i> complex	1 (0.5)	1 (100)	1 (100)	1 (100)
<i>Enterobacter amnigenus</i>	1 (0.5)	0 (0)	0 (0)	1 (100)
<i>Providencia rettgeri</i>	1 (0.5)	1 (100)	1 (100)	NI (100)
<i>Non-Enterobacteriaceae</i>	43 (21.5)	34 (79.1)	33 (76.7)	26 (60)
<i>Pseudomonas aeruginosa</i>	21 (10.5)	21 (100)	21 (100)	21 (100)
<i>Acinetobacter baumannii</i> complex	3 (1.5)	3 (100)	2 (66.7)	3 (100)
<i>Achromobacter xylosoxidans</i>	2 (1)	2 (100)	2 (100)	NI (100)
<i>Stenotrophomonas maltophilia</i>	2 (1)	0 (0)	0 (0)	NI (100)
<i>Bacteroides fragilis</i>	2 (1)	1 (50)	1 (50)	NI (100)
<i>Fusobacterium</i> spp.	2 (1)	0 (0)	0 (0)	NI (100)
<i>Haemophilus influenzae</i>	2 (1)	1 (50)	1 (50)	NI (100)
<i>Acinetobacter lwoffii</i>	1 (0.5)	1 (100)	1 (100)	1 (100)
<i>Acinetobacter junii</i>	1 (0.5)	1 (100)	1 (100)	1 (100)
<i>Aeromonas caviae</i>	1 (0.5)	1 (100)	1 (100)	NI (100)
<i>Alistipes fingoldii</i>	1 (0.5)	0 (0)	0 (0)	NI (100)
<i>Bacteroides thetaiotaomicron</i>	1 (0.5)	0 (0)	0 (0)	NI (100)
<i>Moraxella osloensis</i>	1 (0.5)	1 (100)	1 (100)	NI (100)
<i>Prevotella denticola</i>	1 (0.5)	0 (100)	0 (100)	NI (100)
<i>Prevotella buccae</i>	1 (0.5)	1 (100)	1 (100)	NI (100)
<i>Rhizobium radiobacter</i>	1 (0.5)	1 (100)	1 (100)	NI (100)
Total	200	188 (94)	182 (91)	170 (85)

^aNI, not included in the assay.^bThis includes one false positive sample, identified as *Klebsiella pneumoniae*.

94.9% (149/157) concordance for *Enterobacteriaceae* compared to 76.7% (33/43) for non-*Enterobacteriaceae* isolates ($P < 0.001$). The decline in Sepsityper performance may be attributed to its inability to consistently identify anaerobic Gram-negative bacilli and *Stenotrophomonas maltophilia*. Overall, comparison between the two evaluated assays showed that Sepsityper was able to correctly identified 6% (species level) and 9% (genus level) more Gram-negative bacilli from positive monomicrobial blood cultures than the BC-GN test ($P < 0.01$ and $P < 0.05$, respectively).

An evaluation analysis between these two assays was performed by only including organisms targeted by the BC-GN test (Table 3). Based on this analysis, comparable positive percent agreement (PPA) and negative percent agreement (NPA) were observed between the Sepsityper (96.5% and 98.8%, respectively) and the BC-GN (99.4% and 99.8%, respectively). Of note is that 15% (30/200) of monomicrobial samples were not targeted by the BC-GN test. Among these cultures, the Sepsityper was able to identify 63% (19/30), which represented 9.5% of all monomicrobial cultures.

For detection of drug resistance genes in Gram-negative bacilli, the BC-GN assay detected the CTX-M β -lactamase resistance gene in 3.8% (8/210) of the cultures. Among the isolates detected were *E. coli* ($n = 6$) and *K. pneumoniae* ($n = 2$). The detection of the CTX-M resistance marker in all eight cultures was found to be in

TABLE 3 Targeted evaluation of Verigene BC-GN and MALDI Sepsityper for 170 monomicrobial cultures

Rapid ID test	Definitive ID and resistance marker ^a	No. of cultures	Sensitivity		Specificity	
			TP/(TP + FN) ^b	PPA (95% CI)	TN/(TN + FP) ^b	NPA (95% CI)
Verigene BC-GN	<i>Escherichia coli</i>	82	82/82	100 (96.5–100)	88/88	100 (95.8–100)
	CTX-M	6	6/6	100 (61–100)	164/164	100 (97.7–100)
	<i>Klebsiella pneumoniae</i> ^c	31	30/31	96.8 (83.8–99.4)	139/140	99.3 (96.1–99.9)
	CTX-M	2	2/2	100 (34.2–100)	168/168	100 (97.8–100)
	<i>Pseudomonas aeruginosa</i>	21	21/21	100 (84.5–100)	149/149	100 (97.5–100)
	<i>Proteus</i> spp.	10	10/10	100 (72.3–100)	160/160	100 (97.7–100)
	<i>Enterobacter</i> spp.	17	17/17	100 (81.6–100)	151/153	98.7 (95.4–99.6)
	<i>Acinetobacter</i> spp.	5	5/5	100 (56.6–100)	165/165	100 (97.7–100)
	<i>Klebsiella oxytoca</i>	3	3/3	100 (43.9–100)	167/167	100 (97.8–100)
	<i>Citrobacter</i> spp.	1	1/1	100 (20.7–100)	169/169	100 (97.8–100)
	Total	170	169/170	99.4 (96.7–99.9)	1,188/1,191	99.8 (99.3–99.9)
MALDI Sepsityper	<i>Escherichia coli</i> ^d	82	80/82	97.6 (91.5–99.3)	88/88	100 (95.8–100)
	<i>Klebsiella pneumoniae</i> ^e	31	30/31	96.8 (83.8–99.4)	138/140	98.6 (94.9–99.6)
	<i>Pseudomonas aeruginosa</i>	21	21/21	100 (84.5–100)	149/149	100 (97.5–100)
	<i>Proteus</i> spp. ^f	10	9/10	90 (59.6–98.2)	160/160	100 (97.7–100)
	<i>Enterobacter</i> spp. ^g	17	15/17	88.2 (65.7–96.7)	153/153	100 (97.6–100)
	<i>Acinetobacter</i> spp.	5	5/5	100 (56.6–100)	165/165	100 (97.7–100)
	<i>Klebsiella oxytoca</i>	3	3/3	100 (43.9–100)	157/157	100 (97.6–100)
	<i>Citrobacter</i> spp. ^h	1	1/1	100 (20.7–100)	167/169	98.8 (95.8–99.7)
	Total	170	164/170	96.5 (92.5–98.4)	1,177/1,191	98.8 (98.0–99.3)

^aWith the exception of CTX-M, no other drug resistance markers were detected.

^bTP, true positive; TN, true negative; FP, false positive; FN, false negative.

^cFalse positive sample was BC157; false negative sample was BC172.

^dFalse negative samples were BC61 and BC142.

^eFalse negative sample was BC192; false positive samples were BC04 and BC160.

^fFalse negative sample was BC41.

^gFalse negative samples were BC68 and BC102.

^hFalse positive samples were BC68 and BC102.

agreement with phenotypic antimicrobial susceptibility testing results. No phenotypic evidence of extended-spectrum β -lactamase or carbapenemase production was observed in any of the samples that tested negative for any of the drug resistance genes by BC-GN assay (data not shown).

Discordant result analyses. Discordant BC-GN and Sepsityper results compared to routine culture are shown in Table 4. With a few exceptions, discordant bacterial identification results between routine blood culture and the Sepsityper or BC-GN were resolved with >95% probability by the Vitek 2 system. The identification of *Alistipes fingoldii* (BC91) obtained from routine culture could not be confirmed since the organism is not included in the Vitek 2 ANC card database. In addition, we were not able to differentiate between *Klebsiella pneumoniae* and *Klebsiella variicola* (BC04, BC157, BC160, and BC192) since the Vitek 2 GN card is not capable of differentiating between these two organisms due to similar phenotypic profiles.

BC-GN discrepant results were observed in 2% (4/200) of monomicrobial cultures. Among these, three were identified as *K. pneumoniae* and misidentified by BC-GN as *K. pneumoniae* and *Enterobacter* spp. ($n = 2$) and *K. oxytoca* ($n = 1$). In addition, one positive culture for *K. variicola* was possibly misidentified as *K. pneumoniae*.

For the Sepsityper, we observed 9% (18/200) and 6% (12/200) discordant results at the species and genus levels from monomicrobial cultures. There were 10 cultures that produced invalid scores (i.e., <1.6). These specimens were positive for *P. mirabilis* ($n = 1$), *Prevotella denticola* ($n = 1$), *S. maltophilia* ($n = 2$), *Haemophilus influenzae* ($n = 1$), *Alistipes fingoldii* ($n = 1$), *Fusobacterium* spp. ($n = 2$), *Bacteroides thetaiotaomicron* ($n = 1$), and *Bacteroides fragilis* ($n = 1$). However, only four of these cultures failed to produce any spectra. We also observed three misidentifications in cultures at species-level ID (i.e., ≥ 1.8). Among these, two cultures were positive for *K. variicola* (BC4, BC160) and one was misidentified as *K. pneumoniae* (BC192). Moreover, there were three cultures (BC57, BC61, and BC142) that were concordant at the genus level but failed to provide

TABLE 4 Discordant Verigene BC-GN and MALDI Sepsityper results

Test	Blood culture	Definitive identification	Result(s)
Verigene	BC87	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> , <i>Enterobacter</i> spp.
BC-GN	BC157	<i>Klebsiella variicola</i>	<i>Klebsiella pneumoniae</i>
	BC192	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> , <i>Enterobacter</i> spp.
	BC172	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>
MALDI	BC04	<i>Klebsiella variicola</i>	<i>Klebsiella pneumoniae</i>
Sepsityper	BC41	<i>Proteus mirabilis</i>	ND ^a
	BC56	<i>Prevotella denticola</i>	NIP ^b
	BC57	<i>Acinetobacter baumannii</i> complex	<i>Acinetobacter</i> spp.
	BC61	<i>Escherichia coli</i>	<i>Escherichia</i> spp.
	BC68	<i>Enterobacter amnigenus</i>	<i>Citrobacter koseri</i>
	BC72	<i>Stenotrophomonas maltophilia</i>	NIP
	BC76	<i>Haemophilus influenzae</i>	NIP
	BC91	<i>Alistipes finegoldii</i>	ND
	BC102	<i>Enterobacter cloacae</i> complex	<i>Citrobacter koseri</i>
	BC112	<i>Fusobacterium</i> spp.	NIP
	BC131	<i>Bacteroides thetaiotaomicron</i>	ND
	BC142	<i>Escherichia coli</i>	<i>Escherichia</i> spp.
	BC146	<i>Stenotrophomonas maltophilia</i>	NIP
	BC160	<i>Klebsiella variicola</i>	<i>Klebsiella pneumoniae</i>
	BC162	<i>Fusobacterium</i> spp.	NIP
	BC192	<i>Klebsiella pneumoniae</i>	<i>Klebsiella variicola</i>
	BC193	<i>Bacteroides fragilis</i>	NIP

^aND, not detected.^bNIP, no identification possible (score of <1.6).

species-level ID. Lastly, two cultures generated discrepant ID results, both of which were isolates producing high confidence scores. These two cultures were identified as *Enterobacter amnigenus* (BC68) and *E. cloacae* complex (BC102) by culture and misidentified by the Sepsityper as *Citrobacter koseri*.

Discordant Gram stain results and polymicrobial cultures. A list of blood cultures that shows discordant Gram stain results and/or polymicrobial cultures is shown in Table 5. In this study, polymicrobial cultures accounted for 2.9% (6/210) of the samples (BC43, BC65, BC109, BC133, BC176, and BC196). Gram stain results were incorrectly reported as Gram-negative bacilli in a total of eight samples (BC7, BC43, BC6, BC106, BC109, BC133, BC144, and BC145). Among these samples, Gram-variable rods were reported incorrectly as Gram-negative rods in a total of four cultures (BC7, BC106, BC144, and BC145). The Sepsityper correctly identified three of these cultures at the species level (BC7, BC106, and BC145). In contrast, the BC-GN was not able to detect any of these isolates. In addition, five blood cultures (BC43, BC65, BC109, BC133, and BC176) showed a mixture of Gram-negative and Gram-positive bacteria on routine blood cultures. Overall, the Sepsityper provided species-level ID for one of the organisms in nine of these samples. The BC-GN was able to identify at least one organism in five polymicrobial cultures (BC43, BC65, BC109, BC133, and BC196). For one culture (BC196), the BC-GN successfully identified both Gram-negative organisms while the Sepsityper was only capable of identifying one organism.

DISCUSSION

The methods evaluated in our study have similarities, limitations, and strengths that are important to understand before considering their implementation in the clinical microbiology laboratory. Similarly, the two methods share the advantage of providing rapid identification of Gram-negative bacilli from positive blood cultures and may therefore directly influence the treatment of patients with sepsis and reduce hospital cost by shortening time to adjustment of antimicrobial therapy and decreasing length

TABLE 5 Evaluation of Verigene BC-GN and MALDI Sepsityper for polymicrobial and discordant Gram stain samples

Blood culture	Gram stain ^a	Definitive Identification	Verigene BC-GN result	MALDI Sepsityper result
BC7	GNR	<i>Lysinibacillus fusiformis</i>	NI ^b	<i>Lysinibacillus fusiformis</i>
BC43	GNR	<i>Acinetobacter lwoffii</i>	<i>Acinetobacter</i> spp.	ND ^c
		<i>Staphylococcus epidermidis</i>	NI	<i>Staphylococcus epidermidis</i>
BC65	GNR	<i>Neisseria flavescens</i>	NI	<i>Neisseria flavescens</i>
		<i>Haemophilus paraphrohaemolyticus</i>	NI	ND
		<i>Streptococcus viridans</i> group	NI	ND
BC106	GNR	<i>Paenibacillus thiaminolyticus</i>	NI	<i>Paenibacillus thiaminolyticus</i>
BC109	GNR	<i>Staphylococcus hominis</i>	NI	<i>Staphylococcus hominis</i>
		<i>Veillonella</i> spp.	NI	ND
BC133	GNR	<i>Streptococcus agalactiae</i>	NI	ND
		<i>Proteus mirabilis</i>	<i>Proteus</i> spp.	<i>Proteus mirabilis</i>
		<i>Providencia stuartii</i>	NI	ND
BC144	GNR	<i>Clostridium clostridioforme</i>	NI	NIP ^d
BC145	GNR	<i>Clostridium bolteae</i>	NI	<i>Clostridium bolteae</i>
BC176	GNR	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>	<i>Enterobacter aerogenes</i>
		<i>Kluyvera</i> spp.	NI	ND
		<i>Enterococcus faecium</i>	NI	ND
BC196	GNR	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>
		<i>Escherichia coli</i>	<i>Escherichia coli</i>	ND

^aBlood culture Gram stain result; GNR, Gram-negative rods.^bNI, not included in the assay.^cND, not detected.^dNIP, no identification possible (score of <1.6).

of stay in the hospital in patients with Gram-negative bacteremia (14–18). Nevertheless, while antibiotic modification can be made based on organism identification alone for some bacteria, the antimicrobial resistance marker detection capability of BC-GN would allow for more immediate and targeted medical management decisions in this group of patients (16, 19, 20). Understanding the prevalence of extended-spectrum β -lactamase (ESBL)-harboring or carbapenem-resistant *Enterobacteriaceae* within the population tested by an individual laboratory is also an important factor to consider. In settings with a low prevalence, the Sepsityper may offer a cost-effective solution (~\$5 to \$9 materials cost per test) while the use of the more expensive BC-GN may be cost-effective (~\$60 to \$80 materials cost per test) in settings with a higher prevalence (19, 20). It should be pointed out that the proper utilization and benefits associated with the use of this type of rapid diagnostic test are largely dependent on the effectiveness of an antibiotic stewardship program (3, 5, 14, 17, 18).

Moreover, the time from processing to identification with the BC-GN assay is approximately 2.5 h but requires minimal hands-on time of approximately 5 min per sample. In contrast, the Sepsityper workflow takes around 30 min of hands-on time from processing to identification, depending on the number of samples being processed. This is a significant amount of time that may affect the laboratory's workflow depending on the number of personnel assigned to blood culture processing, their level of proficiency, workload, and other factors unique to each laboratory environment. Furthermore, the BC-GN is limited to the identification of nine Gram-negative bacilli targets. In contrast, the Sepsityper is untargeted and offers a much broader capability for detection and identification of organisms at the genus and species levels. Another key point is that the robustness of the Sepsityper is dependent on its bacterial extraction performance from positive blood cultures and the number of organisms included in the MALDI Biotyper system library. Therefore, clinical microbiology laboratories with the ability to validate the broader research use only MALDI Biotyper library will benefit the most when using the Sepsityper assay compared with those using the more limited U.S. FDA-approved MALDI Biotyper library. In addition, the robustness of the Sepsityper kit is also dependent on the use of the MBT Sepsityper software module, which uses lower log(score) cutoff values than those recommended for Biotyper

identification from solid media. The implementation of these lower cutoff values is due to the “dilution effect” of blood culture-derived peaks, such as hemoglobin, serum, and leukocyte proteins, that reduce log(score)s for organisms isolated from blood cultures compared to the log(score)s obtained from agar plates (11). Lastly, the BC-GN is an U.S. FDA-approved *in vitro* diagnostic test while the Sepsityper is not. This will require those laboratories that intend to use the Sepsityper to expend a significant amount of time, effort, and resources to validate this test and ensure regulatory compliance. In essence, the selection of either test evaluated in this study must be weighed carefully against some of the factors discussed and those unique to each particular laboratory environment.

In this study, a total of 210 positive blood cultures appearing monomicrobial at the Gram stain for Gram-negative bacilli were analyzed. Among these, 95% were monomicrobial blood cultures and the remaining 5% were polymicrobial or showed discrepant Gram stain results. Discrepant Gram stain results were mainly due to the inability to detect Gram-positive organisms in mixed cultures or the presence of Gram-variable organisms (i.e., *Lysinibacillus fusiformis*, *Paenibacillus thiaminolyticus*, *Clostridium* spp.) in monomicrobial cultures, highlighting another limitation of the BC-GN and similar rapid identification panels. As discussed by Buchan et al., automated identification systems require correct interpretation of Gram stain results for their proper utilization (13). Misinterpretation of the Gram stain can lead to a failed identification, which further extends turnaround time and may lead to added expenses. Overall, the proportions of the most common Gram-negative bacilli observed in this study were similar to those reported in large multicenter cohort studies and are also representative of all blood cultures routinely analyzed at this institution (21).

The bacterial identification discrepancies observed in our study between routine culture and Sepsityper or BC-GN assays can be attributed to several factors. In two samples (BC87 and BC192), the BC-GN assay exhibited a positive response for both *K. pneumoniae* and *Enterobacter* spp., but these samples were identified as *K. pneumoniae* only by routine culture. These results may have occurred due to differences in analytical sensitivity between routine culture and the BC-GN test (16). Therefore, it is possible that the conventional culture only isolated *K. pneumoniae* due to the insufficient number of *Enterobacter* spp. in the blood culture. Moreover, the BC-GN assay cross-reactivity limitation of the capture probes has been recognized in other evaluation studies (14–16, 22). This limitation may have accounted for the discrepancies observed in two blood culture samples where the BC-GN identified *K. oxytoca* instead of *K. pneumoniae* (BC172 and BC176) and *E. cloacae* complex and *E. amnigenus* were misidentified as *C. koseri* (BC68 and BC102). Furthermore, a reliable ID may have been compromised by the quality of the specimen or due to the few differences in the proteomic profiles within each group in those cases where no spectral profile or no reliable ID was attained. Lastly, as noted in previous studies, the discrepancies observed in multiple blood culture samples (BC04, BC157, BC160, and BC192) were probably due to the MALDI-TOF MS and Vitek inability to differentiate between *K. pneumoniae* and *K. variicola* due to similar biochemical and proteomic profiles (17, 22, 23). With regard to the BC-GN, it seems more likely that this assay does not misidentify *K. variicola* isolates and correctly reports them as not identified since the BC-GN *K. pneumoniae* probe has a low enough sequence identity with *K. variicola* (88% versus 100% for *K. pneumoniae*) to select against *K. variicola* as a target (22, 23). Regardless, we cannot rule out the possibility of probe cross-reactivity between these two species. The use of 16S rRNA sequencing is not suitable to distinguish between these two species due to their genetic relatedness (17, 23). However, sequencing of the *yggE* gene has been successfully used to differentiate these two species (23). Due to study limitations, we were not able to resolve any of these discrepancies by sequencing of the *yggE* gene.

The evaluation of the Sepsityper and BC-GN assays showed similar excellent performances in the ID of Gram-negative bacilli when only including organisms targeted by the BC-GN test. However, it is important to note that 15% of the bacteria identified from monomicrobial cultures was not targeted by the BC-GN test. This proportion of

less frequent Gram-negative bacteria can fluctuate in different settings. As shown in a previous database analysis of 59 hospitals in the United States, the distribution of pathogens can be significantly different among BSI subgroups (i.e., community-acquired BSI [CAB], health care-associated BSI [HCAB], and hospital-acquired BSI [HAB]) (24). In this study, a statistically significant higher proportion of Gram-negative bacteria was observed for the CAB group compared to that of the HCAB and HAB groups. Although Sepsityper performance showed a diminished performance among non-*Enterobacteriaceae* samples, mainly anaerobic Gram-negative bacilli and *Stenotrophomonas maltophilia*, we suggest that the use of the Sepsityper may further improve clinical outcomes and reductions in health care costs in clinical laboratories where a significant number of “off target” organisms cannot be rapidly identified due to the limitations of the rapid ID panel tests, like the BC-GN.

This study has several limitations. First, there was a lack of specificity data, as only positive blood cultures were examined. Second, we were unable to assess the reproducibility of the Sepsityper results since only a single spot was analyzed for each specimen. Third, we were not able to confirm either the presence of antimicrobial resistance markers by PCR or the identity of discordant ID results by sequencing. Fourth, due to the small number of pediatric bottles used in the study, we were unable to determine its influence in the performance of the evaluated methods. Fifth, although a large number of monomicrobial cultures were included in the study, the Sepsityper analytical accuracy for the less frequent Gram-negative bacilli could not be estimated. Nevertheless, our results demonstrate excellent performance for the ID of the most frequently encountered Gram-negative bacilli, similar to that of the BC-GN test. Lastly, due to the limitations in our study design, we were not able to determine the impact of the evaluated methods in the clinical decision-making process, particularly in those cases where the BC-GN assay was not able to identify any organism or the Gram stain was misinterpreted.

In conclusion, the two methods evaluated in this study showed similar excellent performance characteristics for the ID of the organisms most commonly isolated from blood cultures. Nevertheless, the Sepsityper offers a cost-effective approach with a broader ID range capability that may be standardized for clinical routine use in laboratories. The use of the Sepsityper may further improve clinical management and antimicrobial stewardship in patients with BSIs caused by less frequent Gram-negative bacilli.

MATERIALS AND METHODS

Specimens. Blood cultures were collected in BD Bactec aerobic (Plus + Aerobic/F and Peds Plus/F) and anaerobic (Lytic/10 Anaerobic/F) blood culture bottles as part of the routine standard of care and incubated on a Bactec FX instrumented blood culture system (BD Diagnostics, Sparks, MD). Bactec bottles that contained Gram-negative bacilli from 210 unique patients enrolled between November 2016 and January 2017 were included in the study. Positive blood culture bottles were processed and stored for 1 week at room temperature according to routine laboratory procedures. Specimens were used in accordance with procedures approved by the Koelus Institutional Review Board (study 1607730977A001).

Standard MALDI-TOF MS identification procedure. An aliquot of positive blood specimen was inoculated on appropriate culture medium and incubated. Aerobic cultures were incubated between 18 to 24 h in a 5% CO₂ incubator, and anaerobic cultures were incubated for at least 48 h in an anaerobic chamber or until sufficient growth was present to proceed with testing. Following incubation, isolates were identified using the Bruker Microflex LT MALDI-TOF MS system (Bruker Daltonics, Billerica, MA). A thin film of bacteria taken from a single colony was applied directly onto an individual spot on a 48-spot polished steel target plate using a wooden applicator and allowed to dry. If a single colony was unable to supply enough material to cover the spot, multiple smaller colonies with identical colony morphologies were used. For each plate, 1 μ l of α -cyano-4-hydroxycinnamic acid (HCCA) matrix was included as a negative-control sample and 1 μ l of bacterial test standard (BTS) dissolved in organic solvent (consisting of 50% acetonitrile and 2.5% trifluoroacetic acid) was included as a calibrator and positive control. With the exception of the negative control, each spotted sample was overlaid with 1 μ l of HCCA matrix and allowed to dry. Once dry, the target plate was inserted into the Bruker Microflex LT MALDI-TOF MS system for analysis using the MALDI Biotyper (MBT) Compass software (v4.1) according to the manufacturer's recommendations. The log(score) cutoff values used for organism identification (ID) were as follows: ≥ 2.0 was considered reliable for species-level ID, 1.7 to 1.99 was considered reliable for genus-level ID, and < 1.7 was considered to be no reliable ID.

Verigene Gram-negative blood culture test procedure. The Verigene BC-GN test (Nanosphere, Inc., Northfield, IL) detects *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., and *Acinetobacter* spp. as well as resistance markers (KPC, NDM, CTX-M, VIM, IMP, and OXA). The test was performed in accordance with the manufacturer's recommendations. The BC-GN was processed as bottles signaled positive on every first positive blood culture bottle per single patient episode containing Gram-negative bacilli shortly after routine cultures were performed as part of routine laboratory procedures. External quality controls were run weekly and included a known positive and negative blood sample in accordance with our approved internal quality control plan (IQCP). In brief, a test cartridge, utility tray, and extraction tray were loaded into the Verigene processor SP. A total of 700 μ l of positive blood culture specimen was added to the extraction tray sample well. Nucleic acids from the specimen were extracted and hybridized to microarray. After 2 h, the microarray was transferred to the Verigene reader for analysis. In the event of a "no call" error, the specimen was retested. Following BC-GN analysis, results were immediately reported to the treating clinicians.

Sepsityper processing and MALDI-TOF procedures. Remnant positive blood culture sample obtained from the same bottle used to perform the BC-GN test was processed within 8 h of positive detection using the MALDI Sepsityper kit (Bruker Daltonics GmbH, Bremen, Germany) in accordance with the manufacturer's recommendations (revision D). Briefly, 1.0 ml of the specimen was transferred to a 1.5-ml centrifuge tube (provided). A 200- μ l aliquot of lysis buffer (provided) was added to the blood specimen, and the mixture was vortexed for 15 s prior to centrifugation in an Eppendorf 5415D centrifuge (Eppendorf, Hamburg, Germany) at $16,000 \times g$ for 2 min. Following centrifugation, the supernatant was removed and the bacterial pellet was resuspended in 1.0 ml of wash buffer (provided), vortexed, and centrifuged at $16,000 \times g$ for 1 min. Following centrifugation, the supernatant was discarded, and the pellet was resuspended in 70% ethanol, vortexed, and centrifuged at $16,000 \times g$ for 2 min. The ethanol was discarded, and the pellet was allowed to dry completely. Sequentially, 2 to 50 μ l each of formic acid (70%, vol/vol) and 100% acetonitrile was added to the pellet (depending on pellet size) and thoroughly mixed after each reagent was added. The suspension was centrifuged a final time at $16,000 \times g$ for 2 min, and a 1- μ l aliquot of extracted protein supernatant was transferred to an individual spot on a 48-spot polished stainless steel target plate and allowed to dry. Each spotted samples was overlaid with 1 μ l of HCCA matrix and allowed to dry. Once dry, the target plate was inserted into the Bruker Microflex LT MALDI-TOF MS for analysis using the MBT Sepsityper software module. For the Sepsityper sample, the log(score) cutoff values used for organism ID were as follows: ≥ 1.8 was considered reliable for species-level ID, 1.6 to 1.79 was reliable for genus-level ID, and < 1.6 was considered to be no reliable ID.

Resolution of discordant BC-GN and Sepsityper results. Discrepancies in bacterial identification between routine blood culture and the Sepsityper or BC-GN were arbitrated by the Vitek 2 system (bioMérieux, Inc., Durham, NC, USA) in favor of the last one.

Data analysis. The performances of the Sepsityper and BC-GN assays were compared to bacterial colony identification using the MALDI-TOF MS system in two different ways. To determine the number of organisms correctly detected by each assay, the results were interpreted as follows. (i) For the BC-GN, an organism that was appropriately identified was considered to be correctly detected. (ii) For the Sepsityper, an organism that was appropriately identified at the genus or species level was considered to be correctly detected. (iii) For the BC-GN, an organism not included (targeted) in the assay that was not identified or incorrectly identified at the genus or species level was considered to be incorrectly detected. (iv) For the Sepsityper, an organism that was not identified or incorrectly identified at the genus or species level was considered to be incorrectly detected. For the targeted evaluation that included only organisms targeted by the BC-GN test, the following results were considered to be correctly identified: (i) an organism detectable by the test method that was appropriately detected (i.e., true positive) or (ii) an organism not detectable by the test method that was appropriately not detected (i.e., true negative). For the Sepsityper test, concordance with culture results was calculated to both the genus and species levels based on the following results: (i) an organism appropriately identified at the genus level (score of > 1.6) or (ii) an organism identified appropriately at the species level (score of > 1.8). Concordance with routine culture methods and standard MALDI was calculated as the number of correct organism IDs divided by the total number of organisms identified. The positive percent agreement (PPA) and negative percent agreement (NPA) of the BC-GN and Sepsityper assays were compared to routine culture methods with standard MALDI. The analysis included only organisms targeted by the BC-GN test. The JMP statistical software, v13.1.0 (SAS Institute, Inc., Cary, NC, USA), was used to calculate the 95% confidence interval (95% CI) of performance estimates, and Fisher's exact probability test was used to determine the significance of performance differences observed between the BC-GN and Sepsityper assays. A *P* value of < 0.05 was used as the cutoff for significance.

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The authors declare that there are no conflict of interests regarding the publication of this paper.

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