

# Clinical Evaluation Comparing the BD Phoenix™ Automated Microbiology System with the MicroScan® WalkAway for Identification and Antimicrobial Susceptibility Testing of *Staphylococcus aureus* and *Enterococcus* species

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## ABSTRACT

**BACKGROUND.** The BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD), a new automated system for rapid identification (ID) and antimicrobial susceptibility testing (AST) of clinically relevant bacteria, was compared with the MicroScan® Walkaway-40 System (Dade- Behring MicroScan, West Sacramento, CA) for accuracy of ID and AST results when testing *Staphylococcus aureus* and *Enterococcus* species.

**METHODS.** A total of 202 clinical isolates were compared including 102 *S. aureus* (52 methicillin resistant [MRSA]), 45 *E. faecium* (41 vancomycin resistant [VRE]), 53 *E. faecalis*, and one each of *E. casseliflavus* and *E. gallinarum*. All isolates were inoculated to both MicroScan® Dried GP Breakpoint Combo 20 and BD Phoenix™ PMIC/ID-33 panels and tested according to manufacturer's instructions. All *S. aureus* were also inoculated to oxacillin (6 µg/mL) agar plates and all enterococci to motility medium.

**RESULTS.** The overall rate of agreement between the two systems for species level ID was 100% for *S. aureus* and 99% for *Enterococcus* species. One VRE was identified by MicroScan® as *E. durans/hirae* (99.99%) and by molecular testing as *E. faecium*, in agreement with the Phoenix™ system. Overall, 2628 antimicrobial/organism combinations were evaluated. The rate of categorical agreement between both systems was 96.9% for *S. aureus* (1384 of 1428) and 95.2% for *Enterococcus* species (1142 of 1200). In 89 of the 102 disagreements, the categorical difference was considered minor. One MRSA was missed by each system, while both systems identified all VRE. The Phoenix™ system time-to-results for MRSA detection was a medium of 12.25 h (range, 7.75 to 16 h) and for VRE 10.5 h (range, 7 to 15.75 h). MicroScan® required 16 to 24 h to detect for each.

**CONCLUSION:** These results showed the BD Phoenix™ system to be reliable for both ID and AST testing under clinical conditions for common gram-positive isolates.

## INTRODUCTION

Organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) are a significant threat for disease, particularly in a healthcare environment due to the risk of nosocomial transfer among patients (1, 3, 9, 10). To prevent transmission, empiric treatment is often considered, pending the results of primary testing. Hospitalized or institutionalized individuals who are infected or colonized with MRSA or VRE are generally placed in the appropriate isolation to control transmission and therapeutic treatment revised to target the sensitivities of the specific pathogen (1, 7). Turnaround time for the identification and for antimicrobial sensitivity results is critical to control these resistant organisms (2, 3).

The BD Phoenix™ Automated Microbiology System (Becton Dickinson, Sparks MD) is a new automated system for rapid identification and antimicrobial susceptibility of clinically relevant bacteria. This system was released in September 2004 for implementation into the clinical microbiology lab. The testing process utilizes a duplex susceptibility test methodology using both a turbidity and oxidation-reduction indicator. These methods are employed in doubling antimicrobial concentrations which measures minimum inhibitory concentrations (MIC) at 20 minute intervals during the testing of panels (3). The MicroScan® Walkaway-40 System (Dade-Behring, West Sacramento, CA) which is a widely used automated comparator, evaluates a test panel by measuring turbidity for breakpoint values at the end of fixed time incubation for categorical interpretation (6).

This study was a side-by-side evaluation of the Phoenix™ to the MicroScan® Walkaway-40 comparator for identification and antimicrobial susceptibility testing when evaluating *Staphylococcus aureus* and *Enterococcus* species. As a part of the evaluation, turnaround time was also considered.

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## MATERIALS AND METHODS

**Clinical Samples:** A total of 102 *S. aureus* and 100 *Enterococcus* species from 24 hour subcultures on 5% sheep blood agar, were evaluated. The following study inclusion criteria were used for *S. aureus*; 1) catalase positive using hydrogen peroxide method, 2) Staphaurex™ (Remel, Lenexa, KS) positive for the coagulase enzyme, and 3) gram stain are positive cocci in clusters from wounds or sterile body sites. The inclusion criteria for *Enterococcus* were; 1) pyrrolidonyl peptidase positive as determined by PYR (L-pyrrolidonyl-β-naphthylamide) disk (Remel), 2) gram-positive cocci in chains or pairs from sterile body sites (blood or body fluids and tissue biopsy), or 3) grew on either Columbia CNA agar containing 6 µg/mL vancomycin (Remel) or were alpha hemolytic streptococci that grew on CVA agar containing 10 µg/mL vancomycin (Remel) from stool specimens. The study isolates were tested for routine clinical identification and susceptibility on the MicroScan® Dried GP Breakpoint Combo 20 panel loaded onto the MicroScan® Walkaway-40 System (Dade Behring, West Sacramento, CA) following manufacturer's directions. All isolates were also tested on the BD Phoenix™ PMIC/ID-33 panel loaded on the BD Phoenix™ Automated Microbiology System (Becton Dickinson, Sparks, MD) following manufacturer's directions. *S. aureus* isolates were also tested using MRSA screen agar (Mueller Hinton with 6 µg/mL oxacillin, Remel) spotted with a sterile 10 µL loop simultaneously from inoculum broth for Phoenix™ panel setup and incubated for 24 hours at 37°C. *Enterococcus* isolates were inoculated to motility test medium (Motility Medium S, BD, Sparks, MD) also from Phoenix™ panel setup subculture and incubated at 30°C for 24 hours.

**Data comparison:** Both the identification and susceptibility data from the Phoenix™ and MicroScan® reports were compared using a limited application of BD's inhouse data analysis tool (Marketing Trial Data Entry v4.0.0, Becton Dickinson). Categorical interpretations were included for the following antimicrobial agents: 1) for both organism groups ampicillin, chloramphenicol, clindamycin, erythromycin, gentamycin, levofloxacin, linezolid, penicillin G, rifampin, tetracycline, and vancomycin; 2) for *Staphylococcus* only gatifloxacin, oxacillin, and trimethoprim/sulfamethoxazole, and 3) for *Enterococcus* only quinipristin/dalfopristin and streptomycin. Essential agreement was not considered due to the breakpoint format of the MicroScan® panel used in clinical conditions. Turn-around-time performance for resistance detection were calculated manually by review of time stamps on Phoenix™ reports and the 24-hour hold for MicroScan® automation programming following NCCLS recommendations (6).

**Discrepant Isolates:** Discrepancies were considered when identifications differed or there was a major or very major difference in susceptibility. Major error is defined as resistant by test method (Phoenix) and sensitive by comparator (MicroScan); very major error is defined as sensitive by test method (Phoenix) and resistant by comparator (MicroScan). All susceptibility discrepant isolates were repeat tested in duplicate on each system.

Persistent sensitivity discrepancies from comparisons generating a major error or very major error were inoculated to a confirmatory plate method. Minor errors, defined as either intermediate susceptibility by test method and sensitive or resistant by comparator, or as intermediate by comparator and sensitive or resistant by test method, were noted for categorical disagreement without further workup. Disparity in identification was resolved using a molecular identification based on sequence analysis following amplification testing (5).

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## RESULTS

Species level identification between the two systems demonstrated an overall agreement of 100% (102/102) for *S. aureus* and 99% (99/100) for *Enterococcus* species (44/45 *E. faecium*, 53/53 *E. faecalis*, 1/1 *E. casseliflavus*, and 1/1 *E. gallinarum*). One *Enterococcus* isolate demonstrating vancomycin resistance was identified by MicroScan as *E. durans/hirae* (99.99% probability) and *E. faecium* by Phoenix (99% probability). Subsequent molecular analysis identified the organism as *E. faecium*. Two of the *Enterococcus* isolates included in the study group were determined to be motile by the tube method and were identified by both instruments as *E. casseliflavus* and *E. gallinarum*.

For antimicrobial susceptibility testing, 2628 antimicrobial/organism combinations were evaluated overall. Categorical agreement between both systems was 96.9% for *S. aureus* (1384 of 1428) (Table 1) and 95.2% for *Enterococcus* species (1142 of 1200) (Table 2). In 95.5% (42 of 44) of the *Staphylococcus* disagreements and 81% (47 of 58) of the *Enterococcus* disagreements, the categorical difference was considered a minor error and no further evaluations were performed.

Two of the thirteen discrepancies were in *S. aureus* for oxacillin. A resistant isolate was detected each by one system against a susceptible reading. Results from repeats of both panel types in duplicate as well as screen agar with 6 µg/mL of oxacillin were sensitive for both discrepant isolates (Table 3). Overall, both systems detected 51 of the 52 MRSA included in the study.

Eleven of the thirteen discrepancies were for *Enterococcus* isolates (Table 4). Gentamicin-synergy represented six of these discrepancies and, further, three of those required confirmation growth on gentamicin agar screen plate containing 500 µg/mL to resolve. Two of the discrepant interpretations were generated via the automated application of rules generated by the Phoenix™ BDXpert System software for panel reports. The remaining three discrepancies were found in individual penicillin, tetracycline, and streptomycin-synergy results. In all, 41 of 41 (100%) VRE were detected by each system.

The Phoenix™ system time-to-results was calculated manually at quarter hour gradations from start/end times stamps on electronic reports. For MRSA detection, final test results were available at a median of 12.25 h (range, 7.75 to 16 h). For VRE detection, final test results were available at a median of 10.5 h (range, 7 to 15.75 h). MicroScan® required a full 24 h hold of the panel on the instrument to detect for each, although resistant isolates may have been read and released at 16 h.

Table 1. Comparative agreement of BD Phoenix™ to MicroScan® Overnight panels for *in vitro* susceptibility testing of *Staphylococcus aureus* (n = 102)

Antibiotic	Categorical Interpretation			Categorical Agreement (%)	Categorical Disagreement <sup>a</sup>		
	S	I	R		Minor	Major	Very Major
Ampicillin	9	1	92	99	1	0	0
Chloramphenicol	102	0	0	92	8	0	0
Clindamycin	72	1	29	100	0	0	0
Erythromycin	40	0	62	100	0	0	0
Gatifloxacin	77	13	12	80	20	0	0
Gentamycin	101	0	1	100	0	0	0
Levofloxacin	65	11	26	88	12	0	0
Linezolid	102	0	0	100	0	0	0
Oxacillin	50	0	52	98	0	1	1
Penicillin G	9	0	93	100	0	0	0
Rifampin	100	1	1	99	1	0	0
Tetracycline	99	0	3	100	0	0	0
Trimeth/Sulfa	101	0	1	100	0	0	0
Vancomycin	102	0	0	100	0	0	0
<b>Total Overall</b>	<b>1029</b>	<b>27</b>	<b>372</b>	<b>96.9</b>	<b>42</b>	<b>1</b>	<b>1</b>

Abbreviations: S, susceptible; I, intermediate; R, resistant; Trimeth/Sulfa, Trimethoprim/Sulfamethoxazole.

<sup>a</sup> Minor: Phoenix = Intermediate, MicroScan = Sensitive or Resistant; or Phoenix = Sensitive or Resistant, MicroScan = Intermediate

Major: Phoenix = Resistant, MicroScan = Sensitive

Very Major: Phoenix = Sensitive, MicroScan = Resistant

Table 2. Comparative agreement of BD Phoenix™ to MicroScan® Overnight panels for *in vitro* susceptibility testing of *Enterococcus* species (n = 100)

Antibiotic	Categorical Interpretation			Categorical Agreement (%)	Categorical Disagreement <sup>a</sup>		
	S	I	R		Minor	Major	Very Major
Ampicillin	58	0	42	98	1	1	0
Chloramphenicol	92	0	8	99	1	0	0
Erythromycin	8	18	74	85	15	0	0
Gentamicin -Syn	67	0	33	94	0	4	2
Levofloxacin	35	0	65	98	2	0	0
Linezolid	100	0	0	89	11	0	0
Penicillin G	58	0	42	99	0	1	0
Quin/Dalf	45	4	51	86	13	1	0
Rifampin	62	15	23	100	0	0	0
Streptomycin - Syn	67	0	33	99	0	1	0
Tetracycline	33	2	65	96	3	1	0
Vancomycin	58	1	41	99	1	0	0
<b>Total Overall</b>	<b>683</b>	<b>40</b>	<b>477</b>	<b>95.2</b>	<b>47</b>	<b>9</b>	<b>2</b>

Abbreviations: S, susceptible; I, intermediate; R, resistant; Quin/ Dalf, Quinupristin/Dalfopristin.

<sup>a</sup> Minor: Phoenix = Intermediate, MicroScan = Sensitive or Resistant; or Phoenix = Sensitive or Resistant, MicroScan = Intermediate

Major: Phoenix = Resistant, MicroScan = Sensitive

Very Major: Phoenix = Sensitive, MicroScan = Resistant

Table 3. Repeat evaluation of discrepant results between BD Phoenix™ and MicroScan® for *Staphylococcus aureus* involving major and very major discrepancies in susceptibility.

Code No.	Discrepancy	Original Result		Repeat Result <sup>a</sup>		Confirmed <sup>b</sup> Result
		Phoenix	Microscan	Phoenix	Microscan	
P547	Oxacillin	S	R	S/S	S/S	S
P557	Oxacillin	R	S	S/S	S/S	S

Abbreviations: S, susceptible; R, resistant.

<sup>a</sup> Performed in duplicate

<sup>b</sup> No growth on oxacillin screen agar containing 6 µg/mL.

Table 4. Repeat evaluation of discrepant results between BD Phoenix™ and MicroScan® involving major and very major discrepancies in susceptibility for *Enterococcus* species.

Code No.	Discrepancy Source	Original Result		Repeat Result <sup>a</sup>		Confirmed <sup>b</sup> Result
		Phoenix	Microscan	Phoenix	Microscan	
P655	Ampicillin	R <sup>c</sup>	S	R/R <sup>c</sup>	S/S	R
P668	Gentamycin-Synergy	R	S	R/R	S/R	R <sup>b</sup>
P673	Gentamycin-Synergy	S	R	S/S	S/S	S
P675	Penicillin	R	S	S/S	S/S	S
P695	Streptomycin-Synergy	R	S	NA <sup>d</sup>	NA <sup>d</sup>	NA <sup>d</sup>
P697	Gentamycin-Synergy	R	S	R/R	R/R	R
P708	Quin/Dalf	R <sup>c</sup>	S	R/R <sup>c</sup>	S/S	R
P727	Gentamycin-Synergy	S	R	S/S	S/S	S
P728	Tetracycline	R	S	R	R	R
P740	Gentamycin-Synergy	R	S	R/R	S/S	R <sup>b</sup>
P748	Gentamycin-Synergy	R	S	S/R	S/S	R <sup>b</sup>

Abbreviations: S, susceptible; R, resistant; NA, not available.

<sup>a</sup> Performed in duplicate.

<sup>b</sup> Growth on gentamycin agar screen plate containing 500 µg/mL.

<sup>c</sup> Interpretation was applied by software based on NCCLS recommendations (6).

<sup>d</sup> Testing not available.

### CONCLUSIONS

- The BD Phoenix™ System time to results for MRSA and VRE detection was 5.5 to 11.75 h sooner than MicroScan® results.
- The BD Phoenix™ System gave comparable results to MicroScan® for the identification of both *Staphylococcus aureus* and *Enterococcus* species under clinical conditions.
- The BD Phoenix™ System gave comparable interpretive results to MicroScan® for susceptibility testing of *Staphylococcus aureus* and *Enterococcus* species.