

Evaluation of BD Phoenix Automated System and MicroScan Walkaway System for the Identification and Susceptibility Testing of Clinically Significant Gram-Positive Organisms

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REVISED ABSTRACT

The rapid identification and accurate susceptibility testing of gram-positive organisms is an important function of the clinical microbiology laboratory. The Phoenix (BD Diagnostics, Sparks, MD) is a new rapid automated system for the identification and susceptibility testing of clinically relevant bacteria with a novel panel inoculation system. The purpose of the study was to compare the Phoenix and the MicroScan (Dade Behring, Sacramento, CA) systems for the identification and susceptibility testing of clinically significant gram-positive organisms. A total of 398 isolates (138, *S. aureus*, 102 coagulase negative staph, 107 *Enterococcus* spp., 22 *S. pneumoniae* and 29 other *Streptococcus* spp.) were tested. Overall the Phoenix and MicroScan systems identified 381/398 (95.7%) and 357/376 (94.9%) of the isolates to the species level, respectively. Phoenix and MicroScan identified 137/138 (99.3%) and 138/138 (100%), respectively of the *S. aureus* to the species level; 88/102 (86.3%) and 93/102 (91.2%), respectively of coagulase negative staph to the species level; 107/107 (100%) and 103/107 (96.3%), respectively of *Enterococcus* spp. to the species level; and 29/29 (100%) and 23/29 (79.3%), respectively of *Streptococcus* spp. to the species level. In addition the Phoenix identified 20/22 (90.9%) of *S. pneumoniae* to the species level. Overall the average time to a reportable result (ID and AST) was 12.5 hr and 23.3 hr for the Phoenix and MicroScan, respectively. The Phoenix produced reportable results an average of 6.3 hr, 13.2 hr and 12.6 hr faster than MicroScan for *S. aureus*, coagulase negative staph and *Enterococcus* spp., respectively. Phoenix and MicroScan category agreement were 98.5% and 98.5%, respectively for all antibiotics tested. Phoenix had 68 minor errors, 16 major errors and 0 very major errors. MicroScan had 59 minor errors, 4 major errors and 18 very major errors. In conclusion, the Phoenix and MicroScan systems performed equally well for the identification and susceptibility testing of gram-positive organisms; however the Phoenix system produced results significantly faster than the MicroScan.

INTRODUCTION

Gram-positive organisms especially gram-positive cocci of the genera *Staphylococcus*, *Enterococcus* and *Streptococcus* are considered significant community-acquired and nosocomial pathogens. In addition the continued increase in antimicrobial resistance among these organisms has challenged the Clinical Microbiology Laboratory to produce timely and accurate susceptibility testing results. Thus the rapid and accurate identification (ID) and antimicrobial susceptibility testing (AST) of these organisms is imperative for optimal patient care. Over the past 20 years several automated systems have been developed for the ID and AST of these organisms. The BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) is a newly released instrument for the rapid ID and AST of clinically significant organisms. The ID portion of the panel utilizes conventional, chromogenic and fluorogenic biochemical tests. Phoenix AST is broth-based microdilution utilizing a redox indicator to detect organism growth. Panels are incubated and read by the instrument at 20 minute intervals. The Phoenix also has an expert system for AST interpretation. The Phoenix offers potential advantages compared to other systems including no reagent additions and no need to perform off line tests. The purpose of this study was to compare Phoenix and the MicroScan Walkaway SI (Dade Behring, Sacramento, CA) for their ability to accurately perform ID and AST of clinically significant gram-positive cocci. In addition, the time required for panel inoculation and a completed result (ID and AST) were also assessed for both systems.

MATERIALS AND METHODS

Bacterial isolates. A total of 398 isolates of the genera *Staphylococcus* (240 isolates), *Enterococcus* (107 isolates), *Streptococcus* (50 isolates) and *Aerococcus* (1 isolate) were investigated. Of these strains, 369 were non-duplicate fresh patient isolates and 28 (22 *S. pneumoniae*, 6 *Enterococcus* spp.) were frozen isolates. Fresh isolates were tested directly or subcultured once onto sheep blood agar (BA) (Becton Dickinson and Co., Sparks, MD) to confirm purity. Frozen isolates were subcultured twice onto BA prior to testing.

Phoenix ID/AST. Phoenix gram-positive combination ID/AST panels (PMIC/ID-33) were used for testing *Staphylococcus* spp., *Enterococcus* spp. and *Aerococcus* spp. *Streptococcus* ID/AST investigational panels (SMIC/ID-3) were used for testing *S. pneumoniae* and other *Streptococcus* spp. Organisms were prepared and inoculated according to manufacturer's instructions. Phoenix ID broth was inoculated with colonies from a pure culture equivalent to a 0.5 McFarland standard using a CrystalSpec nephelometer (BD). After transfer of 25 µL of the suspension to the Phoenix AST broth, the remaining suspension was poured into the ID portion of the panel. The Phoenix AST broth was supplemented with one drop of AST indicator (oxidation-reduction indicator) prior to addition of ID broth suspension. After addition of the ID broth suspension the tube was inverted several times to mix. The AST broth was then poured into the AST portion of the panel. Panels were sealed with a plastic closure and panels were logged and loaded into the Phoenix instrument. *Streptococcus* panels were inoculated in the same fashion with the following exception: The Phoenix AST-S broth was supplemented with one drop of AST-S indicator (oxidation-reduction indicator) prior to addition of ID broth suspension.

MicroScan ID/AST. MicroScan gram-positive combination ID/AST panels (PC21) were used for testing *Staphylococcus* spp., *Enterococcus* spp., *Aerococcus* spp. and *Streptococcus* spp. other than *S. pneumoniae* (ID only). *Streptococcus* AST panels (MICroSTREP PLUS Type 1) were used for performing AST on *S. pneumoniae* and other *Streptococcus* spp. Organisms were prepared and inoculated according to manufacturer's instructions. Organism suspensions were prepared using the MicroScan prompt inoculation system. Suspensions were then poured into the RENOK disposable inoculator tray and panels were inoculated using the RENOK. Panels were covered and then logged and loaded into the MicroScan Walkaway SI instrument. MICroSTREP panels were inoculated as follows: Colonies were emulsified in 3 mL of Inoculum Saline to a turbidity equivalent to a 0.5 McFarland standard. Tubes were vortexed and 100 µL of suspension was transferred into 25 mL of MicroScan lysed horse blood broth. Following inversion suspensions were then poured into the RENOK disposable D-inoculator tray and panels were inoculated using the RENOK. Panels were covered and incubated at 35°C in an ambient air incubator for 20-24 hours. MICroSTREP panels were read manually using indirect light.

Timing studies. Timing studies were performed on all organisms tested on ten separate days with each system. The studies were structured to measure the time necessary to prepare panels, prepare inoculum, inoculate panels, enter instrument data and load panels into each instrument. For MicroScan this included the following: panel preparation-open panel, label panel; inoculum preparation-inoculate prompt; panel inoculation-remove RENOK inoculator tray from packaging, pour suspension into seed trough, inoculate panel with RENOK, label and inoculate sterility plate; enter instrument data-enter necessary information into computer; load panels-cover panel and load into instrument. For Phoenix this included the following: panel preparation-open panel and place on rack, label panel, place ID and AST broths in rack, add indicator to AST broth; inoculum preparation-inoculate ID broth using nephelometer, inoculate 25 µL ID broth into AST broth; inoculate panels-add contents of ID and AST broths to panel, cap panel, label and inoculate sterility plate; enter instrument data-enter necessary information into computer; load panels-load panel into instrument.

The total time for each system to produce a final identification and susceptibility results was also recorded.

Quality control. Quality control for each system was performed as recommended by the manufacturer. Each lot of MicroScan gram-positive combo panels was tested using *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 for ID/AST and *S. bovis* ATCC 49147 and *M. luteus* ATCC 49732 for ID. MICroSTREP panels were tested each day of use with *S. pneumoniae* ATCC 49619. Phoenix gram-positive panels were tested each day of use with *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 and Strep panels were tested each day of use with *S. pneumoniae* ATCC 49619.

Data analyses. The ID results from the MicroScan and Phoenix were compared for genus and species agreement. If both systems agreed at the genus and species level, the ID was considered correct. When the genus and/or species were not in agreement the isolate was retested in both systems. If a discrepancy remained it was resolved using conventional and rapid biochemical tests (catalase, coagulase, PYR, 6.5% NaCl, bile esculin, bile solubility), serologic tests (Pneumoslide, streptococcus typing reagents) and commercial identification systems (API Staph, API Strep, BioMérieux, Durham, NC). The ID obtained by biochemical testing was considered the correct ID.

Only those antibiotics tested in both systems were evaluated and category agreement (CA) calculated. Isolates that did not display CA in both systems were repeated. If a discrepancy remained the isolate was retested using disk diffusion (BD Diagnostics, Sparks, MD) (gatifloxacin, gentamicin synergy, nitrofurantoin, norfloxacin, streptomycin synergy) or gradient diffusion (Etest, AB Biodisk, Piscataway, NJ) (ceftriaxone, clindamycin, erythromycin, gentamicin, linezolid, levofloxacin, meropenem, oxacillin, rifampin, trimethoprim/ sulfamethoxazole, vancomycin). Errors were classified as very major error (VME) – false susceptible MicroScan or Phoenix result, major error (ME) – false resistant MicroScan or Phoenix result, and minor error (mE) – MicroScan or Phoenix intermediate susceptibility and reference method susceptible or resistant, or reference method intermediate susceptibility and MicroScan or Phoenix susceptible or resistant.

RESULTS

1. A total of 398 isolates (138, *S. aureus*, 102 coagulase negative staph, 107 *Enterococcus* spp., 22 *S. pneumoniae* and 29 other *Streptococcus* spp.) were tested.
2. The average time to prepare and inoculate panels and load them into their respective instrument was 120 sec/isolate for MicroScan and 162 sec/isolate for Phoenix (Table 1).
3. Overall the Phoenix system identified 381/398 (95.7%) of the isolates to the species level (Table 2). Phoenix identified:
 - a. 137/138 (99.3%) of *S. aureus* to the species level.
 - b. 88/102 (86.3%) of coagulase negative staph to the species level.
 - c. 107/107 (100%) of *Enterococcus* spp. to the species level.
 - d. 29/29 (100%) of *Streptococcus* spp. to the species level.
 - e. 20/22 (90.9%) of *S. pneumoniae* to the species level.
4. Overall the MicroScan system identified 357/376 (94.9%) of the isolates to the species level (Table 2). MicroScan identified:
 - a. 138 /138 (100%) of *S. aureus* to the species level.
 - b. 93/102 (91.2%) of coagulase negative staph to the species level.
 - c. 103/107 (96.3%) of *Enterococcus* spp. to the species level.
 - d. 23/29 (79.3%) of *Streptococcus* spp. to the species level.
5. On the average Phoenix produced more rapid results by 6.3 hr, 13.2 hr, 12.6 hr and 15 hr for *S. aureus*, coagulase negative staph, *Enterococcus* spp. and other *Streptococcus* spp., respectively (Table 3).
6. Susceptibility data were evaluated for 5565 potentially clinically useful isolate-antimicrobial combinations. MicroScan and Phoenix gave comparable results (category agreement) in 5484 (98.5%) and 5481 (98.5%), respectively (Table 4).
 - a. For MicroScan, high mE rates were seen with nitrofurantoin and linezolid vs. *Enterococcus* and high VME rates were seen with trimethoprim/sulfamethoxazole vs. coagulase negative staph.
 - b. For Phoenix, high mE rates were seen with gatifloxacin vs. *S. aureus* and coagulase negative staph.

Table 1. Average time necessary for inoculum preparation, panel inoculation and panel loading into MicroScan and Phoenix.

Task	Average time per panel (sec) [Range]	
	MicroScan	Phoenix
Prepare AST panels	18.6 [14.3-22.6]	34.2 [31.1-41.0]
Prepare inoculum	3.4 [29.1-39.3]	65.0 [55.2-79.1]
Inoculate AST panels	42.8 [38.8-46.8]	42.9 [38.4-54.4]
Enter data into computer	2.9 [9-22.2]	14.9 [10-19]
Load panels into system	10.2 [7.8-14.8]	4.2 [3.3-4.8]
Overall	120 [108-132]	162 [144-174]

Table 2. Identification of gram-positive organisms by MicroScan and Phoenix.

Organism	No. of isolates								
	No. tested	Identified as:							
		Correct species		Correct genus only		Incorrect genus or species		With no identification	
	MS ^a	P ^b	MS	P	MS	P	MS	P	
<i>S. aureus</i>	138	138	137	0	1	0	0	0	0
CNS	102								
<i>S. epidermidis</i>	54	50	45	4	9	0	0	0	0
<i>S. hominis</i>	15	14	15	1	0	0	0	0	0
<i>S. haemolyticus</i>	14	13	14	1	0	0	0	0	0
<i>S. capitis</i>	9	7	7	1	2	0	0	1	0
<i>S. lugdunensis</i>	2	2	2	0	0	0	0	0	0
<i>S. saprophyticus</i>	2	2	2	0	0	0	0	0	0
<i>S. simulans</i>	2	2	2	0	0	0	0	0	0
<i>S. auricularis</i>	1	1	0	0	1	0	0	0	0
<i>S. cohnii</i>	1	1	0	0	0	0	1	0	0
<i>S. warneri</i>	1	1	1	0	0	0	0	0	0
<i>S. xylosus</i>	1	0	0	1	1	0	0	0	0
Enterococcus	107								
<i>E. faecalis</i>	81	80	81	0	0	0	0	1	0
<i>E. faecium</i>	22	19	22	2	0	1	0	0	0
<i>E. avium</i>	1	1	1	0	0	0	0	0	0
<i>E. casseliflavus/gallinarum</i>	1	1	1	0	0	0	0	0	0
<i>E. durans/hirae</i>	1	1	1	0	0	0	0	0	0
<i>E. raffinosus</i>	1	1	1	0	0	0	0	0	0
<i>Streptococcus pneumoniae</i>	22	NT ^c	20	NT	2	NT	0	NT	0
Other Streptococcus									
<i>S. agalactiae</i>	11	11	11	0	0	0	0	0	0
<i>S. pyogenes</i>	15	9	15	6	0	0	0	0	0
Viridans <i>Streptococcus</i>	2	2	2	0	0	0	0	0	0
<i>Aerococcus viridans</i>	1	1	1	0	0	0	0	0	0
TOTAL	398	357	381	16	16	1	1	1	0

^a MS = MicroScan

^b P = Phoenix

^c NT = Not tested

Table 3. Average time to obtain final identification and susceptibility testing results for MicroScan and Phoenix.

Organism	Average time to obtain final ID/AST (hr) [Range]	
	MicroScan	Phoenix
<i>S. aureus</i>	18.9 [16-25.5]	12.6 [9-16]
MRSA ^a	16.8 [16-25.5]	12.5 [9.8-16]
MSSA ^b	24 [24]	12.9 [9-15.8]
CNS ^c	27.5 [16.3-48]	14.3 [10-16]
<i>Enterococcus</i>	23.4 [16-42.5]	10.8 [8-16]
VRE ^d	19.8 [16-24]	12.5 [8.3-16]
VSE ^e	24.2 [24-42.5]	10.4 [8-16]
<i>S. pneumoniae</i>	NT ^f	11.7 [9-16]
Other <i>Streptococcus</i>	27.8 [14.5-47.5]	12.8 [9.8-16.5]

^a MRSA = Methicillin resistant *S. aureus*

^b MSSA = Methicillin susceptible *S. aureus*

^c CNS = Coagulase negative *Staphylococcus*

^d VRE = Vancomycin resistant *Enterococcus*

^e VSE = Vancomycin susceptible *Enterococcus*

^f NT = Not tested

Table 4. Performance of MicroScan and Phoenix for AST of gram-positive organisms

System	No. (%) of errors			
	Overall agreement (%)	VME ^a	ME ^b	mE ^c
MicroScan	98.5	8 (0.3)	4 (0.07)	59 (1.1)
Phoenix	98.5	0	16 (0.3)	68 (1.2)

^a VME = Very major error

^b ME = Major error

^c mE = Minor error

SUMMARY AND CONCLUSIONS

The accurate and timely identification of clinically significant gram-positive cocci including methicillin resistant *S. aureus* and vancomycin resistant *Enterococcus* is imperative for appropriate patient management. Both the MicroScan and Phoenix systems offer automated systems for identification and susceptibility testing of clinically significant gram-positive cocci. The purpose of the study was twofold, first to compare the Phoenix and the MicroScan systems for the identification and susceptibility testing of clinically significant gram-positive cocci; second was to compare the time required for panel “set up” and to obtain a final ID and AST result for each system. The observations of the study included:

1. The MicroScan and the Phoenix were comparable in their ability to identify, to the species level, commonly encountered *Staphylococcus*, *Streptococcus* and *Enterococcus* spp. However Phoenix offers the advantage of automated identification and susceptibility testing of *Streptococcus* spp. including *S. pneumoniae* (pending FDA clearance).
2. The Phoenix system produced final results significantly faster than the MicroScan system particularly for methicillin susceptible *S. aureus* and vancomycin susceptible *Enterococcus*.
3. The time required for panel “set up” is slightly higher for the Phoenix system (2.7 min/panel) compared to the MicroScan system (2.0 min/panel).
4. Both MicroScan and Phoenix perform reliable susceptibility testing with a minimum of errors. The Phoenix system did not produce any very major errors.

In conclusion, the Phoenix and MicroScan systems performed equally well for the identification and susceptibility testing of routinely encountered gram-positive cocci; however the Phoenix system produced results significantly faster than the MicroScan. The Phoenix system also offers the potential advantages of automated ID and AST of *S. pneumoniae* and does not require reagent additions or the use of off line tests.