

Performance of BD Phoenix™ in Low Inoculum Mode for the Identification of Frequently Isolated Gram-Positive Cocci

T. DUNK, K. LANDERKIN, G. GAO, A. PATEL, M. YOUNG, J. SINHA, AND J. SALOMON

BD Diagnostics • 7 Loveton Circle • Sparks, MD 21152, USA

REVISED ABSTRACT

OBJECTIVES: Gram-positive cocci from the genera *Staphylococcus*, *Enterococcus*, and *Streptococcus*, make up a significant portion of the frequently isolated bacteria to be identified in the clinical microbiology laboratory. Because the colonies that these organisms form on a primary isolation plate are often few and small, it is not unusual to subculture for next day testing. This is done to ensure enough growth is present to make a suspension equivalent to a 0.5 McFarland standard — generally the minimum required for ID systems. Utilizing a newly developed low inoculum mode and databases, the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, MD, USA) provides the user with an option of running isolates at an inoculum density equivalent to a 0.25 McFarland standard. The panels that are used with the low inoculum mode are the same as those used with the regular system. This study examines identification accuracy of frequently isolated gram-positive cocci using the BD Phoenix System configured to low inoculum mode.

METHODS: Using the new BD PhoenixSpec nephelometer, 730 gram-positive cocci were set up in Phoenix GP panels at an inoculum density of 0.2-0.3 McFarland units and tested in low inoculum mode (P025). The isolates were comprised of 14 species, which were referenced by a combination of conventional testing and the BD Phoenix System set at regular mode (P05).

RESULTS: Accuracy of identification with P025 was determined to be 97.0% (708/730) to the species level and 99.4% (726/730) to the genus level for the isolates tested. For 1.0% (7/730) of the isolates the P025 did not give a final ID but required a supplemental test to separate 2 or 3 organisms listed under instrument ID. Incorrect identification rates were found to be 2.6% (19/730) to species level and 0.1% (1/730) to genus level, and for 0.4% (3/730) of the isolates no identification was obtained.

CONCLUSION: Frequently isolated gram-positive cocci, tested at low inoculum density, were accurately identified in the BD Phoenix System run in the newly developed low inoculum mode.

INTRODUCTION AND OBJECTIVES

Routine gram-positive cocci in the clinical laboratory include *Staphylococcus*, *Enterococcus*, and *Streptococcus* species. The primary isolation plate of these organisms is frequently comprised of a few small isolated colonies, which can lead to an additional subculture plate/day to ensure enough growth to prepare a 0.5 McFarland standard, which is required by most ID systems. The BD Phoenix™ System now offers a low inoculum mode that provides the user with the flexibility of preparing a 0.25 McFarland standard if needed for these cultures. The low inoculum mode uses the same panel as the regular system with newly created unique databases, but utilizes a new battery powered portable BD PhoenixSpec nephelometer to measure bacterial inoculum densities equivalent to McFarland standards 0.10 to 4.50. This evaluation included 730 gram-positive cocci that were tested in Phoenix GP Panels utilizing the low inoculum mode. The strains used in the study were previously identified by conventional reference testing, and/or the BD Phoenix System set at the regular mode.

MATERIALS AND METHODS

TEST STRAINS: The gram-positive taxa included in the database for the Phoenix low inoculum mode (P025) are shown in Table 1. Seven hundred-thirty gram-positive cocci, representing 14 different species, were tested. Several classes of drug resistant strains such as vancomycin-resistant enterococci, methicillin-resistant staphylococci and *Streptococcus pneumoniae*, which were penicillin intermediate or resistant and multi-drug resistant, were included.

MEDIA: Each isolate was sub-cultured twice onto Trypticase™ Soy Agar plates with 5% defibrinated sheep blood (TSA II™, BDDS) and incubated at 35°C in approximately 5% CO₂ for 18 to 20 hours.

PHOENIX™ ID METHOD: The Phoenix (GP) panel contains 45 dried enzymatic and biochemical substrates including 20 fluorogenic, 10 carbon source/inhibitory, 8 fermentation, 5 chromogenic, and 2 miscellaneous substrates, as well as 2 fluorescent control wells (Table 2). All panels were set up according to the recommended procedures and tested in low inoculum mode (P025). The bacterial suspensions were made from TSA II media and adjusted to a 0.20 – 0.30 McFarland reading using the PhoenixSpec nephelometer (Figure 1). The inoculated panels were placed in a Phoenix instrument according to recommended procedures.

IDENTIFICATION REFERENCE METHODS: Conventional biochemicals and the Phoenix system, run in the regular mode (P05), were used to reference all strains tested in the study.

Figure 1. BD PhoenixSpec Nephelometer



RESULTS

Of the 730 gram-positive cocci tested, 708 (97%) were correctly identified to the species level and 726 (99.4%) were correctly identified to the genus level using P025. Seven of the 730 (1.0%) required a supplemental test, or tests, to separate 2 or 3 organisms listed under instrument ID. There were 19 (2.6%) incorrect identifications to the species level and 1 (0.1%) to the genus level, while 3 out of 730 (0.4%) isolates gave no identification. Table 3 shows the identification accuracy results, broken down by species tested. The misidentifications obtained are shown in Table 4.

The cumulative time to results for the low inoculum mode (P025) is shown in Figure 2. The average time to results was 4.2 hours.

Table 1. Gram-Positive Taxa List for P025

<i>Aerococcus urinae</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus schleiferi</i> ssp <i>coagulans</i>
<i>Aerococcus viridans</i>	<i>Micrococcus lylae</i>	<i>Staphylococcus schleiferi</i> ssp <i>schleiferi</i>
<i>Alloioicoccus otitidis</i>	<i>Pediococcus acidilactici</i>	<i>Staphylococcus sciuri</i>
<i>Dermacoccus nishinomiyaensis</i>	<i>Pediococcus damnosus</i>	<i>Staphylococcus simulans</i>
<i>Enterococcus avium</i>	<i>Pediococcus dextrinicus</i>	<i>Staphylococcus vitulinus</i>
<i>Enterococcus casseliflavus</i>	<i>Pediococcus parvulus</i>	<i>Staphylococcus warneri</i>
<i>Enterococcus durans</i>	<i>Pediococcus pentosaceus</i>	<i>Staphylococcus xylosus</i>
<i>Enterococcus faecalis</i>	<i>Rothia mucilaginosa</i>	<i>Streptococcus agalactiae</i>
<i>Enterococcus faecium</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus anginosus</i>
<i>Enterococcus gallinarum</i>	<i>Staphylococcus auricularis</i>	<i>Streptococcus bovis</i>
<i>Enterococcus hirae</i>	<i>Staphylococcus capitis</i>	<i>Streptococcus bovis</i> I
<i>Enterococcus raffinosus</i>	<i>Staphylococcus caprae</i>	<i>Streptococcus bovis</i> II
<i>Gemella haemolysans</i>	<i>Staphylococcus carnosus</i>	<i>Streptococcus constellatus</i>
<i>Gemella morbillorum</i>	<i>Staphylococcus chromogenes</i>	<i>Streptococcus cristatus</i>
<i>Globicatella sanguinis</i>	<i>Staphylococcus cohnii</i> ssp <i>cohnii</i>	<i>Streptococcus equi</i>
<i>Helcococcus kunzii</i>	<i>Staphylococcus cohnii</i> ssp <i>urealyticum</i>	<i>Streptococcus gordonii</i>
<i>Kocuria kristinae</i>	<i>Staphylococcus epidermidis</i>	<i>Streptococcus group</i> C/G
<i>Kocuria rosea</i>	<i>Staphylococcus equorum</i>	<i>Streptococcus intermedius</i>
<i>Kocuria varians</i>	<i>Staphylococcus felis</i>	<i>Streptococcus mitis</i>
<i>Kytococcus sedentarius</i>	<i>Staphylococcus gallinarum</i>	<i>Streptococcus mutans</i>
<i>Lactococcus lactis</i> ssp <i>cremoris</i>	<i>Staphylococcus haemolyticus</i>	<i>Streptococcus oralis</i>
<i>Lactococcus lactis</i> ssp <i>hordniae</i>	<i>Staphylococcus hominis</i>	<i>Streptococcus parasanguinis</i>
<i>Lactococcus plantarum</i>	<i>Staphylococcus hyicus</i>	<i>Streptococcus pneumoniae</i>
<i>Leuconostoc citreum</i>	<i>Staphylococcus intermedius</i>	<i>Streptococcus porcinus</i>
<i>Leuconostoc lactis</i>	<i>Staphylococcus kloosii</i>	<i>Streptococcus pyogenes</i>
<i>Leuconostoc mesenteroides</i> ssp <i>mesenteroides</i>	<i>Staphylococcus lentus</i>	<i>Streptococcus salivarius</i>
<i>Listeria innocua</i>	<i>Staphylococcus lugdunensis</i>	<i>Streptococcus sanguinis</i>
<i>Listeria monocytogenes</i>	<i>Staphylococcus pasteurii</i>	<i>Streptococcus sobrinus</i>
<i>Macrocococcus caseolyticus</i>	<i>Staphylococcus saprophyticus</i>	<i>Streptococcus uberis</i>
		<i>Streptococcus vestibularis</i>

Table 2. Phoenix Gram-Positive Panel Layout

LOC	SUB-CODE	LOC	SUB-CODE	LOC	SUB-CODE
A1	R_BGEN	B1	R_MTT	C1	R_DTAG
A2	R_DSUC	B2	R_NGU	C2	R_MAL
A3	C_DGUA	B3	R_DTRE	C3	R_DEX
A4	C_3MGA	B4	M_BDCEL	C4	A_ARARR
A5	C_DFRU	B5	A_LALT	C5	A_GLPRB
A6	C_IMN	B6	M_BDGLU	C6	M_BDGLC
A7	C_CLST	B7	A_LPLOB	C7	A_LLEUH
A8	C_PXB	B8	A_LPYR	C8	M_NAG
A9	C_KGA	B9	FLR_CTL	C9	FLR_CTL
A10	C_DMNT	B10	A_LPHET	C10	A_LARGH
A11	C_MAA	B11	A_LTRY	C11	M_PHOT
A12	C_THY	B12	M_PHOS	C12	A_LHIST
A13	P_PAGLU	B13	A_META	C13	A_LISO
A14	P_PHOL	B14	M_ADGLU	C14	M_BDGAL
A15	N_VAALA	B15	N_ALALH	C15	S_URE
A16	N_LPROT	B16	T_ESC	C16	
A17		B17		C17	

Figure 2. Cumulative Time to Results for all Isolates Tested

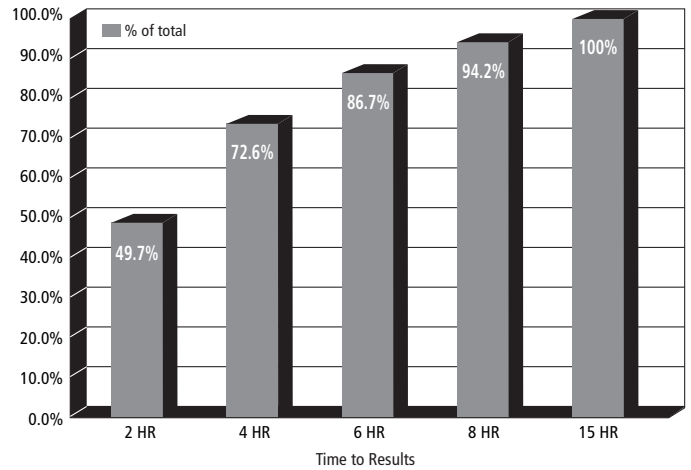


Table 3. Performance Results

ORGANISM	# TESTED	# (%) CORRECT TO SPECIES	# (%) INCORRECT TO SPECIES	# (%) CORRECT TO GENUS	# (%) INCORRECT TO GENUS	# (%) NO IDENTIFICATION
<i>Enterococcus casseliflavus</i>	26	26 (100%)	0	26 (100%)	0	0
<i>Enterococcus faecalis</i>	57	56 (98.2%)	0	56 (98.2%)	0	1 (1.8%)
<i>Enterococcus faecium</i>	79	78 (98.7%)	1 (1.3%)	78 (98.7%)	1 (1.3%)	0
<i>Enterococcus raffinosus</i>	20	20 (100%)	0	20 (100%)	0	0
<i>Staphylococcus aureus</i>	101	101 (100%)	0	101 (100%)	0	0
<i>Staphylococcus epidermidis</i>	92	91 (98.9%)	1 (1.1%)	92 (100%)	0	0
<i>Staphylococcus haemolyticus</i>	50	46 (92.0%)	3 (6.0%)	49 (98.0%)	0	1 (2.0%)
<i>Staphylococcus lugdunensis</i>	20	18 (90.0%)	1 (5.0%)	19 (95.0%)	0	1 (5.0%)
<i>Staphylococcus saprophyticus</i>	33	29 (87.9%)	4 (12.1%)	33 (100%)	0	0
<i>Staphylococcus schleiferi</i>	24	21 (87.5%)	3 (12.5%)	24 (100%)	0	0
<i>Streptococcus agalactiae</i>	34	34 (100%)	0	34 (100%)	0	0
<i>Streptococcus bovis</i>	38	35 (92.1%)	3 (7.9%)	38 (100%)	0	0
<i>Streptococcus pneumoniae</i>	116	114 (98.3%)	2 (1.7%)	116 (100%)	0	0
<i>Streptococcus pyogenes</i>	40	39 (97.5%)	1 (2.5%)	40 (100%)	0	0
TOTAL	730	708 (97.0%)	19 (2.6%)	726 (99.4%)	1 (0.1%)	3 (0.4%)

Table 4. List of Incorrect Identifications

REFERENCE IDENTIFICATION	ID OBTAINED WITH P025
<i>Enterococcus faecium</i>	<i>Streptococcus bovis II</i>
<i>Staphylococcus epidermidis</i>	<i>Staphylococcus schleiferi ssp schleiferi</i>
<i>Staphylococcus haemolyticus</i>	<i>Staphylococcus capitis</i>
<i>Staphylococcus haemolyticus</i>	<i>Staphylococcus caprae</i>
<i>Staphylococcus haemolyticus</i>	<i>Staphylococcus capitis</i>
<i>Staphylococcus lugdunensis</i>	<i>Staphylococcus haemolyticus</i>
<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus kloosii</i>
<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus epidermidis</i>
<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus hominis</i>
<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus auricularis</i>
<i>Staphylococcus schleiferi ssp coagulans</i>	<i>Staphylococcus schleiferi ssp schleiferi*</i>
<i>Staphylococcus schleiferi ssp schleiferi</i>	<i>Staphylococcus schleiferi ssp coagulans*</i>
<i>Staphylococcus schleiferi ssp schleiferi</i>	<i>Staphylococcus schleiferi ssp coagulans*</i>
<i>Streptococcus bovis II</i>	<i>Streptococcus anginosus</i>
<i>Streptococcus bovis II</i>	<i>Streptococcus cricetus</i>
<i>Streptococcus bovis II</i>	<i>Streptococcus gordonii</i>
<i>Streptococcus pneumoniae</i>	<i>Streptococcus mitis</i>
<i>Streptococcus pneumoniae</i>	<i>Streptococcus cricetus</i>
<i>Streptococcus pyogenes</i>	<i>Streptococcus mitis</i>

* Species misidentified to subspecies level, not species level. These were included as incorrect "species" identification in Table 3.

CONCLUSIONS

n The Phoenix System, set to low inoculum mode, lends flexibility to the clinical microbiology laboratory by allowing testing of colonies which are insufficient in number to achieve a 0.5 McFarland standard. This helps alleviate the need for additional subculturing and provides more timely results to the physician. In this study of frequently encountered gram-positive cocci, the P025 system was found to provide highly accurate identifications.

