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Accuracy of Identification in BD Phoenix™ Using the New Low Inoculum Mode.

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REVISED ABSTRACT

OBJECTIVES: Automated microbiology systems generally require the user to make an inoculum density equivalent to a 0.5 McFarland standard or greater. At times there are an insufficient number of isolated colonies on a primary plate to achieve that concentration without adding a day for subculture. In order to address this concern, a low inoculum mode for the BD Phoenix™ Automated Microbiology System (BD Diagnostics) was developed. The low inoculum mode utilizes the same panels as the regular systems but requires just half of the inoculum density. This study examined identification accuracy when testing routine clinical isolates, as well as less frequently encountered organisms, using the low inoculum mode.

METHODS: A total of 2139 strains, including 1055 gram-positive and 1084 gram-negative bacteria, representing more than 175 taxa, were tested in the BD Phoenix System in low inoculum mode. The strains were identified either by classical conventional tests or by using the BD Phoenix System in regular mode. The user's manual was followed for setting up the panels in regular mode while the new BD PhoenixSpec nephelometer was incorporated to measure an inoculum density range of 0.2 – 0.3 McFarland for panels set up in low inoculum mode.

RESULTS: The identification accuracy for gram-positives was 97.8% correct, 1.3% incorrect and 0.9% no identification. Multiple answers (more than 1 ID listed) were given 3.4% of the time with the average time to results (TTR) at 5.6 hours. The identification accuracy for gram-negatives was 98.1% correct, 1.3% incorrect and 0.6% no identification. Multiple answers were given 1.3% of the time and the average TTR was 4.7 hours.

CONCLUSION: The low inoculum mode of the BD Phoenix System is an acceptable alternative for accurate identification of gram-negative rods and gram-positive cocci while requiring fewer colonies thereby reducing the need for subculture.

INTRODUCTION AND OBJECTIVES

Microbiology laboratories routinely utililize automated systems to identify bacteria in cultures. A requirement of many such systems is to make a broth suspension of the isolate to be identified at a density equivalent to a 0.5 McFarland standard, or 1.5 x 10^8 CFU/mL. In some cases there are not enough colonies of the same morphology in primary culture to achieve this density and subculture is required adding a day to the resolution of the culture. In order to address this delay, a low inoculum mode was developed for the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, MD, USA). The low inoculum mode utilizes the current gram-positive and gram-negative panels but works with a suspension equivalent to a 0.25 McFarland standard, or 7.5 x 10^7 CFU/mL. This study was conducted to determine the identification accuracy, as well as the time-to-results (TTR), for the new low inoculum mode.

MATERIALS AND METHODS

SYSTEM DESCRIPTION: While the new low inoculum mode of the BD Phoenix System utilizes the same panels, new databases and algorithms have been developed allowing for the lower inoculum density. Identifications are completed in a time frame of 2 – 15 hours. The inoculum density modes for gram-positive and gram-negative panels can be configured independently of each other allowing the user to run either panel type at 0.5 or 0.25 McFarland (Figure 1). The user may also run panels in the opposite mode from which the instrument is configured. In a case where the instrument is configured to the 0.5 mode, and the user has insufficient growth on the primary isolation plate to achieve that density, well A17 can be darkened with a permanent black marker such as a Sharpie™ (Figure 2). At this point the panel can be inoculated using a suspension density equivalent to a 0.25 McFarland and the instrument will process it using the low inoculum mode. In the same respect, if the instrument is configured to 0.25 McFarland, the user may run a panel at 0.5 McFarland by darkening well A17.

Figure 1.

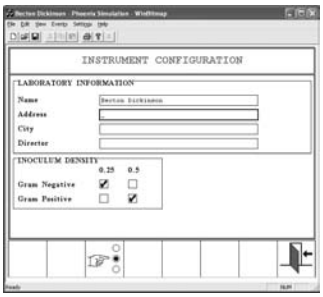
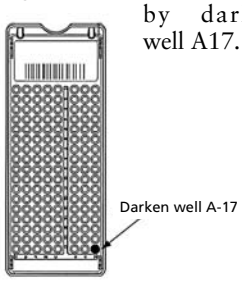


Figure 2.



TESTING: A total of 2139 strains, comprised of 1055 gram-positive and 1084 gram-negative isolates, were tested in the low inoculum mode. The species that were tested are listed in Tables 1 and 2. The strains were identified either by classic conventional tests or by using the BD Phoenix System in the regular mode. The panels were set up per the user's manual for testing with the Phoenix system in regular mode. Testing with the low inoculum mode encompassed the newly developed BD PhoenixSpec nephelometer (Figure 3) for making an inoculum density of 0.20 – 0.30 McFarland. Discrepant results were repeated in duplicate with the 2 out of 3 rule applying as the final ID.

Figure 3. BD PhoenixSpec Nephelometer

Table 1.

Organism Name	# Tested	Organism Name	# Tested
Aerococcus urinae	7	*Staphylococcus haemolyticum*	48
Aerococcus viridans	7	*Staphylococcus hominis*	28
Enterococcus avium	9	*Staphylococcus hyicus*	3
Enterococcus casseliflavus	13	*Staphylococcus intermedius*	3
Enterococcus durans	4	*Staphylococcus kloosii*	4
Enterococcus faecalis	102	*Staphylococcus lentus*	3
Enterococcus faecium	84	*Staphylococcus lugdunensis*	17
Enterococcus gallinarum	8	*Staphylococcus pasteuri*	2
Enterococcus hirae	8	*Staphylococcus saprophyticus*	25
Enterococcus raffinosus	13	*Staphylococcus schleiferi* ssp. *coagulans*	3
Gemella haemolysans	2	*Staphylococcus schleiferi* ssp. *schleiferi*	3
Gemella morbillorum	2	*Staphylococcus sciuri*	7
Globicatella sanguinis	7	*Staphylococcus simulans*	15
Kocuria rosea	3	*Staphylococcus vitulinus*	3
Kocuria varians	1	*Staphylococcus warneri*	10
Lactococcus lactis ssp. *cremoris*	1	*Staphylococcus xylosus*	13
Leuconostoc lactis	2	*Streptococcus agalactiae*	28
Listeria innocua	9	*Streptococcus anginosus*	11
Listeria monocytogenes	19	*Streptococcus bovis*	22
Macrococcus caseolyticus	2	*Streptococcus constellatus*	3
Micrococcus luteus	3	*Streptococcus equi* ssp. *equi*	4
Pediococcus acidilactici	7	*Streptococcus equi* ssp. *zooepidemicus*	3
Pediococcus pentosaceus	3	*Streptococcus gordonii*	3
Rothia mucilaginosus	12	*Streptococcus intermedius*	2
Satphylococcus felis	4	*Streptococcus mitis*	4
Staphylococcus aureus	225	*Streptococcus mutans*	4
Staphylococcus auricularis	5	*Streptococcus oralis*	3
Staphylococcus capitis ssp. *capitis*	14	*Streptococcus parasanguinis*	1
Staphylococcus capitis ssp. *ureolyticus*	7	*Streptococcus pneumoniae*	20
Staphylococcus caprae	1	*Streptococcus porcinus*	3
Staphylococcus carnosus	1	*Streptococcus pyogenes*	24
Staphylococcus chromogenes	1	*Streptococcus salivarius*	5
Staphylococcus cohnii ssp. *cohnii*	2	*Streptococcus sanguinis*	4
Staphylococcus cohnii ssp. *urealyticum*	4	*Streptococcus sobrinus*	3
Staphylococcus epidermidis	119	*Streptococcus uberis*	4
Staphylococcus equorum	2	*Streptococcus vestibularis*	1
Staphylococcus gallinarum	3		

Table 2.

Organism Name	# Tested	Organism Name	# Tested
Achromobacter species	4	*Leclercia adecarboxylata*	2
Acinetobacter baumannii	41	*Leminorella grimontii*	2
Acinetobacter lwoffii	8	*Leminorella richardii*	1
Actinobacillus lignieresii	2	*Moellerella wisconsensis*	2
Actinobacillus suis	1	*Morganella morganii*	27
Actinobacillus ureae	1	*Myroides odoratus / odoratimimus*	2
Aeromonas caviae	3	*Ochrobactrum anthropi*	4
Aeromonas hydrophila	5	*Oligella urethralis*	2
Aeromonas sobria	4	*Pantoea agglomerans*	7
Alcaligenes faecalis	2	*Pasteurella multocida*	3
Bergeyella zoohelcum	2	*Pasteurella pneumotropica*	2
Bodetella bronchiseptica	2	*Plesiomonas shigelloides*	4
Brevundimonas diminuta	3	*Pragia fontium*	2
Brevundimonas vesicularis	2	*Proteus mirabilis*	57
Burkholderia cepacia	18	*Proteus penneri*	2
Burkholderia gladioli	2	*Proteus vulgaris*	16
Cardiobacterium hominis	2	*Providencia alcalifaciens*	1
CDC group EO2	3	*Providencia rettgeri*	14
Cedecea lapagei	2	*Providencia rustigianii*	2
Cedecea neteri	3	*Providencia stuartii*	30
Chromobacterium violaceum	1	*Pseudomonas aeruginosa*	100
Chryseobacterium indologenes	3	*Pseudomonas fluorescens*	4
Chryseobacterium meningosepticum	4	*Pseudomonas luteola*	2
Citrobacter amalonaticus	1	*Pseudomonas mendocina*	1
Citrobacter braakii	6	*Pseudomonas oryzihabitans*	2
Citrobacter freundii	24	*Pseudomonas putida*	7
Citrobacter koseri	22	*Pseudomonas stutzeri*	2
Citrobacter werkmanii	3	*Ralstonia pickettii*	2
Citrobacter youngae	3	*Rhizobium radiobacter*	1
Comamonas testosteroni	2	*Salmonella choleraesuis* ssp. *arizonae*	2
Delftia acidovorans	2	*Salmonella gallinarum*	2
Edwardsiella tarda	4	*Salmonella* species	16
Eikenella corrodens	2	*Salmonella typhi*	5
Empedobacter brevis	1	*Serratia liquefaciens*	2
Enterobacter aerogenes	33	*Serratia marcescens*	26
Enterobacter amnigenus 2	2	*Serratia odorifera* 1	2
Enterobacter asburiae	4	*Serratia plymuthica*	2
Enterobacter cancerogenus	4	*Serratia rubidaea*	2
Enterobacter cloacae	71	*Shewanella putrefaciens*	6
Enterobacter hormaechei	2	*Shigella dysenteriae*	2
Enterobacter sakazakii	3	*Shigella flexneri*	9
Escherichia coli	177	*Shigella sonnei*	14
Escherichia fergusonii	2	*Sphingobacterium multivorum*	4
Escherichia hermannii	2	*Sphingomonas paucimobilis*	2
Escherichia vulneris	1	*Stenotrophomonas maltophilia*	40
Hafnia alvei	7	*Suttonella indologenes*	2
Klebsiella oxytoca	34	*Vibrio hollisae*	2
Klebsiella pneumoniae ssp. *ozaenae*	1	*Vibrio parahaemolyticus*	2
Klebsiella pneumoniae ssp. *pneumoniae*	80	*Weeksella virosa*	2
Klebsiella pneumoniae ssp. *rhinoscleromatis*	1	*Yersinia enterocolitica*	18
Kluyvera ascorbata	2	*Yersinia kristensenii*	2
Kluyvera cryocrescens	1	*Yokenella regensburgei*	2

RESULTS

The identification accuracy and average time-to-results (TTR) are shown in Table 3. For gram-positive organisms the low inoculum mode yielded identification accuracy of 97.8% correct, 1.3% incorrect, and 0.9% no identification. Multiple answers were given 3.4% of the time and the average TTR was 5.6 hours. For gram-negative organisms the low inoculum mode yielded identification accuracy of 98.1% correct, 1.3% incorrect and 0.9 % no identification. Multiple answers were given 1.3% of the time and the average TTR was 4.7 hours. For both the GP and GN systems, misidentified strains were randomly spread throughout the many species tested. The cumulative TTR for each system are shown in Figures 4 and 5.

Table 3. Performance Results.

	# Tested	# (%) Correct	# (%) Incorrect	# (%) Not Identified	Average Time to Results
Gram Positive	1055	1032 (97.8%)	14 (1.3%)	9 (0.9%)	5.6 hours
Gram Negative	1084	1063 (98.1%)	14 (1.3%)	7 (0.6%)	4.7 hours

Figure 4. Cumulative Time to Results For All GP Isolates Tested

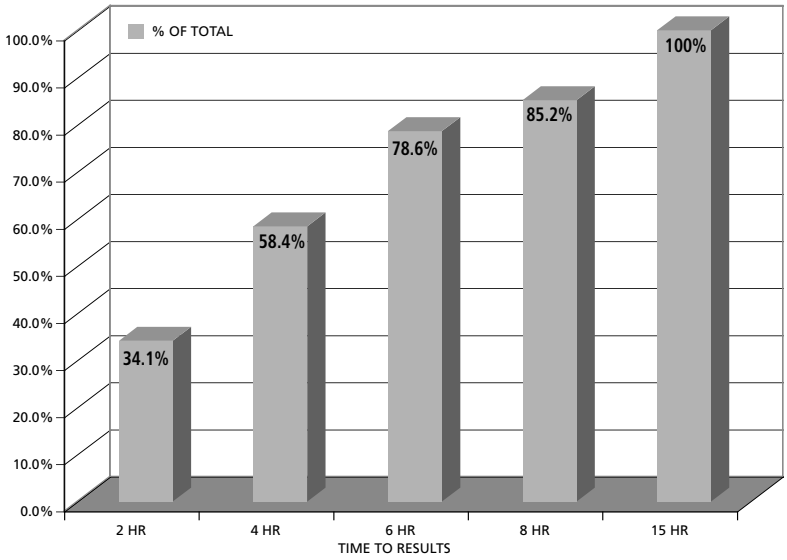
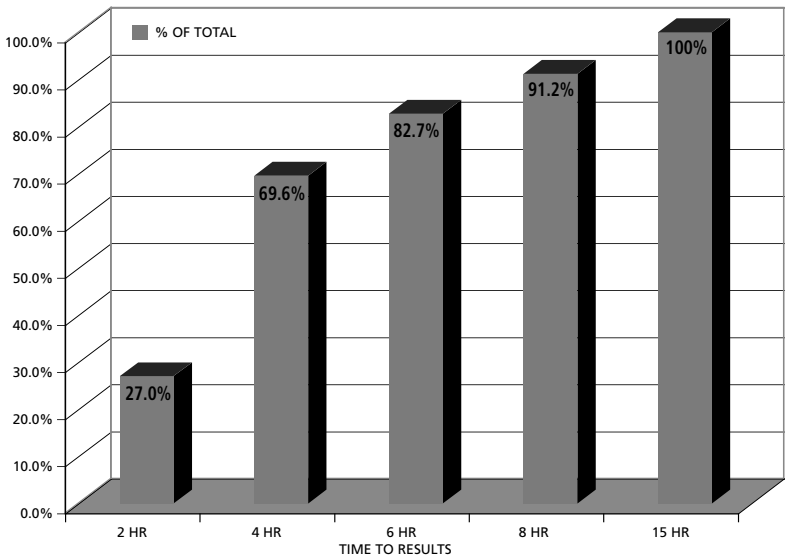


Figure 5. Cumulative Time to Results For All GN Isolates Tested



CONCLUSIONS

The Phoenix System, run in low inoculum mode:

- Provides acceptable identification accuracy of common, and less frequently encountered, gram-positive cocci and gram-negative bacilli.
- Produces rapid time to results.
- Presents flexibility to the microbiology lab in setting up Phoenix panels.
- Helps alleviate the need for subculture, thus providing timely results to the physician.