

# Identification of Gram-Negative Bacilli in BD Phoenix™ Using the New Low Inoculum Mode

J. SALOMON, E. SMITH, T. HANSEN, V. WHITE, T. GRESOCK, W. WILLIAMS, AND J. REUBEN

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

## REVISED ABSTRACT

**OBJECTIVES:** Identification of gram-negative bacilli using automated microbiology systems has been a common and accepted laboratory practice for many years. The systems that provide rapid results typically require the user to make an inoculum concentration that is equivalent to a 0.5 McFarland standard. Occasionally, due to an insufficient number of colonies on the primary isolation media, this inoculum requirement is not readily achieved. In order to address these cases, a low inoculum mode has been developed for the BD Phoenix™ Automated Microbiology System (BD Diagnostics, Sparks, MD, USA). The low inoculum mode utilizes the same panels as the regular system, but contains new databases and only requires an inoculum density equivalent to a 0.25 McFarland standard. This study investigates identification accuracy of the Phoenix System, set to low inoculum mode, for frequently encountered and clinically significant gram-negative bacilli.

**METHODS:** A total of 865 gram-negative bacilli, comprised of 15 genera, were tested in the BD Phoenix System set on low inoculum mode (P025). The majority of strains had been biochemically referenced using classical methods, while a small portion were run in parallel using the BD Phoenix System on regular mode (P05) as the reference. The P05 was set up according to the Users Manual and the P025 was set up using the new BD PhoenixSpec nephelometer at an inoculum density ranging from 0.2 – 0.3 McFarland units.

**RESULTS:** Of the total 865 isolates tested, the P025 correctly identified 838 (96.9%) to the species level and 850 (98.3%) to the genus level. There were 15 cases (1.7%) which listed 2 or 3 choices under instrument ID requiring a supplemental test to produce a final ID. The P025 yielded 18 (2.1%) incorrect results to the species level and 6 (0.7%) to the genus level, while 9 (1.0%) strains resulted in no identification. Average time to results came out as 4.67 hours for the total 865 strains that were tested.

**CONCLUSION:** The newly developed low inoculum mode of the BD Phoenix System provides acceptable performance for the identification of routine and clinically significant gram-negative bacilli while using half of the normal inoculum density.

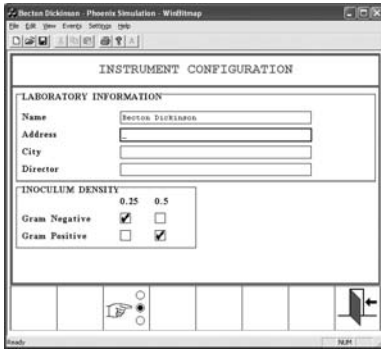
## INTRODUCTION AND OBJECTIVES

The use of automated microbiology systems to identify bacteria has been a widely accepted practice for many years. Over time these automated systems have been improved and updated, providing users with increased taxa claims, quicker time to results, and better accuracy. One of the universal steps in preparing panels for automated systems is making a suspension of the test organism in a given amount of broth. This suspension (or inoculum density) usually has a requirement stipulating equivalence to a 0.5 McFarland standard ( $1.5 \times 10^8$  CF/mL). Occasionally users will encounter circumstances where they do not have enough colonies on a primary isolation plate to achieve this requirement. In these situations the organism is often re-subcultured meaning a delay of 18-24 hours in obtaining results. To address these concerns, a low inoculum mode has been developed for the BD Phoenix™ Automated Microbiology System (BD Diagnostics). The low inoculum mode encompasses current gram-negative and gram-positive Phoenix panels, but only requires a suspension density equivalent to 0.25 McFarland units ( $7.5 \times 10^7$ ). The objective of this study was to determine identification accuracy of the Phoenix system, set to low inoculum mode, with frequently encountered and clinically significant gram-negative bacilli.

## MATERIALS AND METHODS

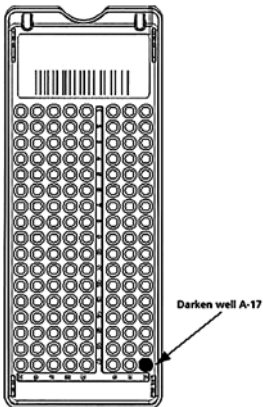
**SYSTEM DESCRIPTION:** The low inoculum mode of the BD Phoenix System (P025) utilizes newly developed algorithms and separate databases, providing identifications in a time frame of 2 – 15 hours. The gram-negative taxa (Table 1) is nearly the same as the BD Phoenix System set on regular mode (P05) – 151 organisms in the P025 and 160 in the P05. Instrument configuration is independent for gram-

Figure 1.



negative (GN) and gram-positive (GP) inoculum settings, allowing the user to run either type panel at 0.5 or 0.25 McFarland (Figure 1). Furthermore, the user may run panels with an inoculum density that is opposite of the configured setting if they so choose. In the event a user is configured to the 0.5 mode and has a plate

Figure 2.



with insufficient colonies to reach that inoculum density, they can simply darken well A17 with a black permanent marker – such as a Sharpie™ (Figure 2). At this point they can set up the panel, using an inoculum density of 0.25 McFarland, and it will be processed as a low inoculum panel. By the same means, an instrument configured to the 0.25 mode can still process a panel that has an inoculum density of 0.5 McFarland as a regular inoculum panel.

**TESTING:** A total of 865 gram-negative bacilli, consisting of 24 species from 15 different genera, were tested in the P025. The majority of isolates were referenced biochemically using classical methods, whereas a small portion were run in parallel using the P05 as the reference. Each isolate was subcultured twice onto TSA w/5% defibrinated sheep blood agar plates (TSA II™ – BD Diagnostics) and incubated at 35°C in 5% CO<sub>2</sub> before testing in Phoenix. The P05 was set up according to the Users Manual, while the P025 incorporated the BD PhoenixSpec nephelometer for measuring inoculum densities. The BD PhoenixSpec (Figure 3) is a newly developed nephelometer that reads in McFarland units out to the hundredths place and has a range of 0.10 – 4.50 McFarland units. The acceptable inoculum density range for the P025 is 0.20 – 0.30.

Figure 3. BD PhoenixSpec Nephelometer



## RESULTS

The P025 was able to correctly identify 838 (96.9%) of the total 865 isolates to the species level and 850 (98.3%) to the genus level. In 15 (1.7%) cases the instrument listed 2 or 3 choices under instrument ID which required a supplemental test(s) to attain a final ID. Incorrect results were produced 18 (2.1%) times to the species level and 6 (0.7%)

times to the genus level. Additionally, 9 (1.0%) isolates yielded a result of no identification. Table 2 provides a breakdown of the organisms tested and Table 3 lists the strains that misidentified. The average time to results for all 865 strains tested was 4.67 hours. Figure 4 presents a graph of the cumulative time to results.

Table 1. Gram-Negative Taxa List for P025

<i>Achromobacter</i> species	<i>Enterobacter amnigenus</i> 1	<i>Pseudomonas stutzeri</i>
<i>Acinetobacter baumannii-calcoaceticus</i> complex	<i>Enterobacter amnigenus</i> 2	<i>Rahnella aquatilis</i>
<i>Acinetobacter haemolyticus</i>	<i>Enterobacter asburiae</i>	<i>Ralstonia pickettii</i>
<i>Acinetobacter Iwoffii</i>	<i>Enterobacter cancerogenus</i>	<i>Raoultella ornithinolytica</i>
<i>Actinobacillus lignieresii</i>	<i>Enterobacter cloacae</i>	<i>Rhizobium radiobacter</i>
<i>Actinobacillus suis</i>	<i>Enterobacter gergoviae</i>	<i>Salmonella choleraesuis</i> ssp. <i>arizonae</i>
<i>Actinobacillus ureae</i>	<i>Enterobacter hormaechei</i>	<i>Salmonella choleraesuis</i> ssp. <i>choleraesuis</i>
<i>Aeromonas caviae</i>	<i>Enterobacter intermedius</i>	<i>Salmonella gallinarum</i>
<i>Aeromonas hydrophila</i>	<i>Enterobacter sakazakii</i>	<i>Salmonella paratyphi</i> A
<i>Aeromonas salmonicida</i> ssp. <i>masoucida</i>	<i>Escherichia coli</i>	<i>Salmonella pullorum</i>
<i>Aeromonas salmonicida</i> ssp. <i>salmonicida</i>	<i>Escherichia fergusonii</i>	<i>Salmonella</i> species
<i>Aeromonas salmonicida</i> ssp. <i>smithia</i>	<i>Escherichia hermannii</i>	<i>Salmonella typhi</i>
<i>Aeromonas schubertii</i>	<i>Escherichia vulneris</i>	<i>Serratia ficaria</i>
<i>Aeromonas sobria</i>	<i>Ewingella americana</i>	<i>Serratia fonticola</i>
<i>Aeromonas veronii</i>	<i>Hafnia alvei</i>	<i>Serratia liquefaciens</i>
<i>Alcaligenes faecalis</i>	<i>Klebsiella oxytoca</i>	<i>Serratia marcescens</i>
<i>Bergeyella zoohelcum</i>	<i>Klebsiella pneumoniae</i> ssp. <i>ozaenae</i>	<i>Serratia odorifera</i> 1
<i>Bordetella bronchiseptica</i>	<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	<i>Serratia odorifera</i> 2
<i>Brevundimonas diminuta</i>	<i>Klebsiella pneumoniae</i> ssp. <i>rhinoscleromatis</i>	<i>Serratia plymuthica</i>
<i>Brevundimonas vesicularis</i>	<i>Kluyvera ascorbata</i>	<i>Serratia rubidaea</i>
<i>Burkholderia cepacia</i>	<i>Kluyvera cryocrescens</i>	<i>Shewanella putrefaciens</i>
<i>Burkholderia gladioli</i>	<i>Leclercia adecarboxylata</i>	<i>Shigella boydii</i>
<i>Cardiobacterium hominis</i>	<i>Leminorella grimontii</i>	<i>Shigella dysenteriae</i>
CDC group EF4a	<i>Leminorella richardii</i>	<i>Shigella flexneri</i>
CDC group EF4b	<i>Mannheimia haemolytica</i>	<i>Shigella sonnei</i>
CDC group EO2	<i>Moellerella wisconsensis</i>	<i>Sphingobacterium multivorum</i>
CDC group Vb3	<i>Morganella morganii</i>	<i>Sphingobacterium spiritovorum</i>
<i>Cedecea davisae</i>	<i>Myroides odoratus / odoratimimus</i>	<i>Sphingobacterium thalpophilum</i>
<i>Cedecea lapagei</i>	<i>Ochrobactrum anthropi</i>	<i>Sphingomonas paucimobilis</i>
<i>Cedecea neteri</i>	<i>Oligella ureolytica</i>	<i>Stenotrophomonas maltophilia</i>
<i>Chromobacterium violaceum</i>	<i>Oligella urethralis</i>	<i>Suttonella indologenes</i>
<i>Chryseobacterium gleum</i>	<i>Pantoea agglomerans</i>	<i>Tatumella ptyseos</i>
<i>Chryseobacterium indologenes</i>	<i>Pasteurella aerogenes</i>	<i>Vibrio alginolyticus</i>
<i>Chryseobacterium meningosepticum</i>	<i>Pasteurella multocida</i>	<i>Vibrio cholerae</i>
<i>Citrobacter amalonaticus</i>	<i>Pasteurella pneumotropica</i>	<i>Vibrio fluvialis</i>
<i>Citrobacter braakii</i>	<i>Photobacterium damsela</i>	<i>Vibrio hollisae</i>
<i>Citrobacter farmeri</i>	<i>Plesiomonas shigelloides</i>	<i>Vibrio metschnikovii</i>
<i>Citrobacter freundii</i>	<i>Pragia fontium</i>	<i>Vibrio mimicus</i>
<i>Citrobacter koseri</i>	<i>Proteus mirabilis</i>	<i>Vibrio parahaemolyticus</i>
<i>Citrobacter sedlakii</i>	<i>Proteus penneri</i>	<i>Vibrio vulnificus</i>
<i>Citrobacter werkmanii</i>	<i>Proteus vulgaris</i>	<i>Wautersia paucula</i>
<i>Citrobacter youngae</i>	<i>Providencia alcalifaciens</i>	<i>Weeksella virosa</i>
<i>Comamonas terrigena</i>	<i>Providencia rettgeri</i>	<i>Yersinia enterocolitica</i>
<i>Comamonas testosteroni</i>	<i>Providencia rustigianii</i>	<i>Yersinia frederiksenii</i>
<i>Delftia acidovorans</i>	<i>Providencia stuartii</i>	<i>Yersinia intermedia</i>
<i>Edwardsiella hoshinae</i>	<i>Pseudomonas aeruginosa</i>	<i>Yersinia kristensenii</i>
<i>Edwardsiella ictaluri</i>	<i>Pseudomonas fluorescens</i>	<i>Yersinia pseudotuberculosis</i>
<i>Edwardsiella tarda</i>	<i>Pseudomonas luteola</i>	<i>Yersinia ruckeri</i>
<i>Eikenella corrodens</i>	<i>Pseudomonas mendocina</i>	<i>Yokenella regensburgei</i>
<i>Empedobacter brevis</i>	<i>Pseudomonas oryzihabitans</i>	
<i>Enterobacter aerogenes</i>	<i>Pseudomonas putida</i>	

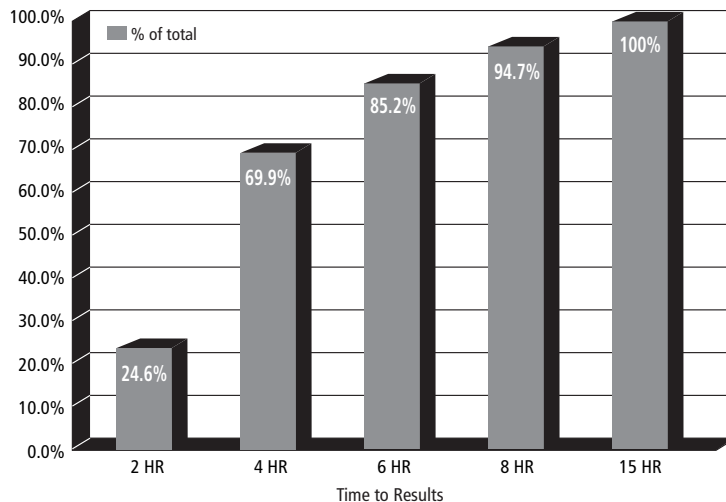
Table 2. Performance Results

ORGANISM	# TESTED	# CORRECT (SPECIES)	# INCORRECT (SPECIES)	# CORRECT (GENUS)	# INCORRECT (GENUS)	# NO ID
<i>Acinetobacter baumannii</i>	25	24 (96.0%)	1 (4.0%)	25 (100%)	0	0
<i>Burkholderia cepacia</i>	45	42 (93.3%)	0	42 (93.3%)	0	3 (6.7%)
<i>Citrobacter freundii</i>	36	32 (88.9%)	3 (8.3%)	35 (97.2%)	0	1 (2.8%)
<i>Citrobacter koseri</i>	20	20 (100%)	0	20 (100%)	0	0
<i>Enterobacter aerogenes</i>	35	34 (97.1%)	1 (2.9%)	34 (97.1%)	1 (2.9%)	0
<i>Enterobacter cloacae</i>	47	42 (89.4%)	3 (6.4%)	43 (91.5%)	2 (4.3%)	2 (4.3%)
<i>Escherichia coli</i>	121	120 (99.2%)	1 (0.8%)	120 (99.2%)	1 (0.8%)	0
<i>Klebsiella oxytoca</i>	39	39 (100%)	0	39 (100%)	0	0
<i>Klebsiella pneumoniae</i>	54	52 (96.3%)	1 (1.9%)	52 (96.3%)	1 (1.9%)	1 (1.9%)
<i>Morganella morganii</i>	35	35 (100%)	0	35 (100%)	0	0
<i>Proteus mirabilis</i>	45	44 (97.8%)	1 (2.2)	45 (100%)	0	0
<i>Proteus vulgaris</i>	27	25 (92.6%)	2 (7.4%)	27 (100%)	0	0
<i>Providencia rettgeri</i>	20	20 (100%)	0	20 (100%)	0	0
<i>Providencia stuartii</i>	23	23 (100%)	0	23 (100%)	0	0
<i>Pseudomonas aeruginosa</i>	73	73 (100%)	0	73 (100%)	0	0
<i>Salmonella choleraesuis</i>	12	12 (100%)	0	12 (100%)	0	0
<i>Salmonella species</i>	23	23 (100%)	0	23 (100%)	0	0
<i>Serratia marcescens</i>	28	27 (96.4%)	1 (3.6%)	28 (100%)	0	0
<i>Shigella boydii</i>	20	19 (95.0%)	1 (5.0%)	20 (100%)	0	0
<i>Shigella dysenteriae</i>	18	16 (88.9%)	1 (5.6%)	16 (88.9%)	1 (5.6%)	1 (5.6%)
<i>Shigella flexneri</i>	25	22 (88.0%)	2 (8.0%)	24 (96.0%)	0	1 (4.0%)
<i>Shigella sonnei</i>	29	29 (100%)	0	29 (100%)	0	0
<i>Stenotrophomonas maltophilia</i>	45	45 (100%)	0	45 (100%)	0	0
<i>Yersinia enterocolitica</i>	20	20 (100%)	0	20 (100%)	0	0
<b>TOTAL</b>	<b>865</b>	<b>838 (96.9%)</b>	<b>18 (2.1%)</b>	<b>850 (98.3%)</b>	<b>6 (0.7%)</b>	<b>9 (1.0%)</b>

Table 3. List of Incorrect Identifications

REFERENCE IDENTIFICATION	ID OBTAINED WITH P025
<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
<i>Citrobacter freundii</i>	<i>Citrobacter braakii</i>
<i>Citrobacter freundii</i>	<i>Citrobacter youngae</i>
<i>Citrobacter freundii</i>	<i>Citrobacter werkmanii</i>
<i>Enterobacter aerogenes</i>	<i>Klebsiella pneumoniae ssp. pneumoniae</i>
<i>Enterobacter cloacae</i>	<i>Klebsiella pneumoniae ssp. pneumoniae</i>
<i>Enterobacter cloacae</i>	<i>Enterobacter sakazakii</i>
<i>Enterobacter cloacae</i>	<i>Klebsiella pneumoniae ssp. pneumoniae</i>
<i>Escherichia coli</i>	<i>Shigella boydii</i>
<i>Klebsiella pneumoniae ssp. pneumoniae</i>	<i>Enterobacter cloacae</i>
<i>Proteus mirabilis</i>	<i>Proteus vulgaris</i>
<i>Proteus vulgaris</i>	<i>Proteus mirabilis</i>
<i>Proteus vulgaris</i>	<i>Proteus mirabilis</i>
<i>Serratia marcescens</i>	<i>Serratia liquefaciens</i>
<i>Shigella boydii</i>	<i>Shigella flexneri</i>
<i>Shigella dysenteriae</i>	<i>Escherichia coli</i>
<i>Shigella flexneri</i>	<i>Shigella boydii</i>
<i>Shigella flexneri</i>	<i>Shigella dysenteriae</i>

Figure 4. Cumulative Time to Results for all Isolates Tested



### CONCLUSIONS

The newly developed low inoculum mode of the BD Phoenix System:

- n Provides acceptable performance for the identification of routine and clinically significant gram-negative bacilli.
- n Requires only half of the inoculum density of the regular mode.
- n Produces rapid time to results.
- n Offers the user flexibility in setting up Phoenix panels.