

# Identification Accuracy of Gram-Negative Bacilli in the BD Phoenix™ Automated Microbiology System

V. WHITE, K. FISCHBEIN, T. DUNK, W. WILLIAMS, J. REUBEN, J. SALOMON

BD Diagnostic Systems • 7 Loveton Circle • Sparks, MD, USA 21152

## ABSTRACT

**OBJECTIVES:** To evaluate the accuracy of the BD Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) in the identification of gram-negative (GN) bacilli from routine clinical specimens, less frequently isolated species, and antimicrobial resistant strains.

**METHODS:** In an internal study, the performance of the Phoenix System was evaluated by comparing the Phoenix Gram-Negative Identification results from three categories of organisms to the results obtained from the Vitek® GNI+ system (bioMérieux, Inc. Hazelwood, MO). Discrepant results were arbitrated using conventional test methodology. The three categories of organisms were comprised of 297 recent clinical isolates, 245 stock isolates including less common species for challenging the breadth of taxa, and 199 stock isolates that exhibit antimicrobial resistance. A total of 93 species representing 49 genera were included in the study. All isolates were subcultured twice before testing and inoculated on each system using the manufacturer's recommended procedure.

**RESULTS:** Combined accuracy of the Phoenix System, for all organisms tested, was determined to be 96.9% (718/741) with 19 strains (2.6%) misidentified and 4 (0.5%) yielding no identification. The accuracy results for the individual categories were 99% (294/297), 95.5% (234/245), and 95.5% (190/199) for the recent clinical isolates, less common species, and resistant strains respectively. These three categories also yielded 3 (1%), 8 (3.3%), and 8 (4.0%) misidentified strains and 0 (0%), 3 (1.2%), and 1 (0.5%) strains which provided no identification respectively.

**CONCLUSIONS:** The results of this study demonstrate the ability of the Phoenix System to accurately identify gram-negative bacilli that are routinely encountered in clinical specimens, as well as less frequently encountered species, and resistant strains.



## INTRODUCTION

The BD Phoenix Automated Microbiology System, (BD Diagnostic Systems, Sparks, MD), is a newly developed automated identification (ID) and susceptibility test system. The gram-negative identification segment utilizes 45 biochemicals to identify aerobic and facultative anaerobic gram-negative bacilli between 2 and 12 hours. The instrument monitors visible and fluorescent spectral changes at 20 minute intervals, interprets and provides an answer when it is confident in the ID. This evaluation was a comprehensive test of the ability of the Phoenix Automated Microbiology System to identify 741 gram-negative bacilli. Three categories of specimens were included: recent clinical isolates, stock and less frequently isolated species, and antimicrobial resistant strains.

## MATERIALS AND METHODS

**TEST STRAINS:** A total of 741 gram-negative isolates were tested: 297 recent clinical isolates obtained from local hospitals and clinical laboratories, 245 stock isolates including less common species obtained worldwide, and 199 resistant isolates composed of strains from the Centers for Disease Control and Prevention (Atlanta, GA), and La Societe Francaise de Microbiologie (French National Reference Center Collection, Paris, France).

**MEDIA:** Each test isolate was sub-cultured twice onto Trypticase™ Soy agar plates with 5% defibrinated sheep blood (TSA II,™ BDDS) and incubated at 35±1°C for 18–20 h in ambient air. The Phoenix and Vitek® (bioMerieux, Inc., Hazelwood, MO) systems were inoculated from the same plate.

**PHOENIX ID METHOD:** The Phoenix ID System contains 45 dried enzymatic and biochemical substrates that include 13 fluorogenic, 16 fermentation, 9 carbon source, 4 chromogenic, 3 miscellaneous substrates, and 2 fluorescent control wells. All panels were set up according to the manufacturer's recommended procedures. A bacterial suspension from the culture on TSA II was prepared in Phoenix ID broth and adjusted to a 0.5–0.6 McFarland reading in using the CrystalSpec™ Nephelometer (BDDS). A final inoculation density of approximately 1.5x10<sup>8</sup> cfu/ml was used. The inoculated panels were placed into the Phoenix instrument for incubation and continuous reading.

**VITEK 1 ID METHOD:** The Vitek GNI+ Card containing 28 substrates, 1 negative control and 1 growth control was used. All cards were set up and processed according to the manufacturer's recommended procedures.

**ARBITRATION METHODS:** When discrepancies between Phoenix and Vitek occurred the isolate was repeated in duplicate in both systems. If the systems were still in disagreement after repeat testing, then conventional methodology and biochemicals were used to determine the final identification.

## RESULTS AND DISCUSSION

Table 1. Overall Identification Accuracy

Category	# Tested	Genus Level Correct	Species Level Correct
Recent	297	296 (99.6%)	294 (99.0%)
Stock/Less Common	245	240 (98.0%)	234 (95.5%)
Resistant	199	194 (97.5%)	190 (95.5%)
<b>Total</b>	<b>741</b>	<b>730 (98.5%)</b>	<b>718 (96.9%)</b>

Table 2. Misidentified Strains

Category	Study No.	Reference ID	Phoenix ID
Recent	B352A	<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
	B637A	<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
	B639A	<i>Pantoea agglomerans</i>	<i>Klebsiella pneumoniae ssp. pneumoniae</i>
Stock/Less Common	B094A	<i>Serratia odorifera</i>	<i>Serratia liquefaciens</i>
	B157A	<i>Yersinia kristensenii</i>	<i>Yersinia ruckeri</i>
	B159A	<i>Yersinia frederiksenii</i>	<i>Yersinia intermedia</i>
	B184A	<i>Myroides odoratus/odoratimimus</i>	<i>Burkholderia cepacia</i>
	B203A	<i>Sphingobacterium multivorum</i>	<i>Sphingobacterium spiritivorum</i>
	B238A	<i>Brevundimonas vesicularis</i>	<i>Weeksella virosa</i>
	B270A	<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
Resistant	B272A	<i>Sphingobacterium multivorum</i>	<i>Sphingobacterium spiritivorum</i>
	B046A	<i>Ochrobactrum anthropi</i>	<i>Ralstonia paucula</i>
	B801A	<i>Klebsiella oxytoca</i>	<i>Klebsiella pneumoniae ssp. pneumoniae</i>
	B814A	<i>Klebsiella pneumoniae ssp. pneumoniae</i>	<i>Klebsiella pneumoniae ssp. ozaenae</i>
	B835A	<i>Acinetobacter baumannii</i>	<i>Acinetobacter lwoffii</i>
	B892A	<i>Enterobacter cloacae</i>	<i>Citrobacter farmeri</i>
	B902A	<i>Escherichia coli</i>	<i>Shigella boydii</i>
	B903A	<i>Enterobacter cloacae</i>	<i>Enterobacter asburiae</i>
	B905A	<i>Burkholderia cepacia</i>	<i>Achromobacter species</i>

Table 3. Strains Yielding No ID in Phoenix

Category	Study #	Organism Name
Stock/Less Common	B208A	<i>Bordetella bronchiseptica</i>
Stock/Less Common	B298A	<i>Acinetobacter lwoffii</i>
Stock/Less Common	B325A	<i>Sphingomonas paucimobilis</i>
Resistant	B883A	<i>Providencia stuartii</i>

Table 4. Recent Isolates

Organism Name	Total Number	Number Correct	Number Incorrect	Number of No ID
<i>Acinetobacter baumannii</i>	7	5	2	
<i>Acinetobacter baumannii/calcoaceticus complex</i>	2	2		
<i>Acinetobacter haemolyticus</i>	1	1		
<i>Alcaligenes faecalis</i>	2	2		
<i>Brevundimonas diminuta</i>	1	1		
<i>Citrobacter freundii</i>	5	5		
<i>Citrobacter koseri</i>	2	2		
<i>Enterobacter aerogenes</i>	14	14		
<i>Enterobacter cloacae</i>	24	24		
<i>Escherichia coli</i>	84	84		
<i>Hafnia alvei</i>	1	1		
<i>Klebsiella oxytoca</i>	3	3		
<i>Klebsiella pneumoniae ssp. pneumoniae</i>	37	37		
<i>Moraxella osloensis</i>	1	1		
<i>Morganella morganii</i>	4	4		
<i>Pantoea agglomerans</i>	1	0	1	
<i>Proteus mirabilis</i>	35	35		
<i>Providencia stuartii</i>	2	2		
<i>Pseudomonas aeruginosa</i>	46	46		
<i>Salmonella species</i>	1	1		
<i>Serratia marcescens</i>	6	6		
<i>Stenotrophomonas maltophilia</i>	18	18		
<b>Total</b>	<b>297</b>	<b>294</b>	<b>3</b>	

Table 5. Stock/Less Common Isolates

Organism Name	Total Number	Number Correct	Number Incorrect	Number of No ID
<i>Achromobacter piechaudii</i>	2	2		
<i>Achromobacter xylosoxidans</i> ssp. <i>xylosoxidans</i>	3	3		
<i>Acinetobacter baumannii</i>	2	1	1	
<i>Acinetobacter lwoffii</i>	3	2		1
<i>Actinobacillus lignieresii</i>	1	1		
<i>Aeromonas hydrophila</i>	4	4		
<i>Agrobacterium radiobacter</i>	4	4		
<i>Alcaligenes faecalis</i>	3	3		
<i>Bordetella bronchiseptica</i>	1	0		1
<i>Brevundimonas diminuta</i>	5	5		
<i>Brevundimonas vesicularis</i>	5	4	1	
<i>Burkholderia cepacia</i>	7	7		
CDC group EF-4a	2	2		
<i>Cedecea davisae</i>	2	2		
<i>Chryseobacterium gleum</i>	2	2		
<i>Chryseobacterium indologenes</i>	1	1		
<i>Chryseobacterium meningosepticum</i>	5	5		
<i>Citrobacter amalonaticus</i>	3	3		
<i>Citrobacter braakii</i>	3	3		
<i>Citrobacter freundii</i>	5	5		
<i>Citrobacter koseri</i>	5	5		
<i>Delftia acidovorans</i>	5	5		
<i>Comamonas testosteroni</i>	3	3		
<i>Edwardsiella tarda</i>	3	3		
<i>Eikenella corrodens</i>	1	1		
<i>Enterobacter aerogenes</i>	4	4		
<i>Enterobacter cloacae</i>	4	4		
<i>Enterobacter gergoviae</i>	4	4		
<i>Enterobacter sakazakii</i>	3	3		
<i>Escherichia fergusonii</i>	1	1		
<i>Escherichia hermannii</i>	3	3		
<i>Hafnia alvei</i>	4	4		
<i>Kingella kingae</i>	1	1		
<i>Klebsiella oxytoca</i>	5	5		
<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	1	1		
<i>Leclercia adecarboxylata</i>	1	1		
<i>Moellerella wisconsinensis</i>	1	1		
<i>Moraxella (Branhamella) catarrhalis</i>	5	5		
<i>Morganella morganii</i>	4	4		
<i>Myroides odoratus/odoratimimus</i>	5	4	1	
<i>Ochrobactrum anthropi</i>	4	4		
<i>Oligella urethralis</i>	1	1		
<i>Pantoea agglomerans</i>	1	1		
<i>Pasteurella multocida</i>	4	4		
<i>Photobacterium damsela</i>	3	3		
<i>Plesiomonas shigelloides</i>	3	3		
<i>Proteus mirabilis</i>	3	3		
<i>Proteus vulgaris</i>	3	3		
<i>Providencia alcalifaciens</i>	3	3		
<i>Providencia rettgeri</i>	3	3		
<i>Providencia stuartii</i>	3	3		
<i>Pseudomonas aeruginosa</i>	6	6		
<i>Pseudomonas luteola</i>	3	3		
<i>Pseudomonas oryzae</i>	3	3		
<i>Pseudomonas putida</i>	3	3		
<i>Pseudomonas stutzeri</i>	1	1		
<i>Pseudomonas violaceum</i>	2	2		
<i>Salmonella choleraesuis</i> ssp. <i>arizonae</i>	2	2		
<i>Salmonella choleraesuis</i> ssp. <i>choleraesuis</i>	2	2		
<i>Salmonella paratyphi</i> A	3	3		
<i>Salmonella</i> species	6	6		
<i>Salmonella typhi</i>	3	3		
<i>Serratia liquefaciens</i>	2	2		
<i>Serratia marcescens</i>	3	3		
<i>Serratia odorifera</i> 1	3	2	1	
<i>Serratia plymuthica</i>	3	3		
<i>Serratia rubidaea</i>	2	2		
<i>Shewanella putrefaciens</i>	3	3		
<i>Shigella flexneri</i>	5	5		
<i>Shigella sonnei</i>	5	5		
<i>Sphingobacterium multivorum</i>	3	1	2	
<i>Sphingomonas paucimobilis</i>	2	1		1
<i>Stenotrophomonas maltophilia</i>	8	8		
<i>Vibrio cholerae</i>	3	3		
<i>Weeksella virosa</i>	3	3		
<i>Yersinia enterocolitica</i>	3	3		
<i>Yersinia frederiksenii</i>	2	1	1	
<i>Yersinia intermedia</i>	1	1		
<i>Yersinia kristensenii</i>	1	0	1	
<i>Yersinia pseudotuberculosis</i>	2	2		
<b>Total</b>	<b>245</b>	<b>234</b>	<b>8</b>	<b>3</b>

The overall identification accuracy for the 741 gram-negative isolates tested was 98.5% (730/741) to the genus level and 96.9% (718/741) to the species level (Table 1). The majority of all Phoenix identifications in this study were obtained between 2 and 4 hours. The accuracy results for the recent clinical isolates to the genus and species level were 99.6% (296/297) and 99.0% (294/297) correct. The accuracy results for the stock and resistant strains to the genus and species level were 98.0% (240/245) and 95.5% (234/245) and 97.5% (194/199) and 95.5% (190/199) correct respectively (Table 2). In this study there were 19 (2.6%) strains that were misidentified, 3 (1%) recent isolates, 8 (3.3%) less common strains, and 8 (4.0%) resistant strains (Table 2). Additionally 4 strains yielded no identification, 3 (1.2%) stock strains and 1 (0.5%) resistant strain (Table 3).

Tables 4, 5, and 6 show the break down of the three categories of organisms tested and the final outcome for each isolate in those groups. Table 7 shows the breakdown of the 741 isolates that were tested by class and the final results obtained.

Table 6. Resistant Isolates

Organism Name	Total Number	Number Correct	Number Incorrect	Number of No ID
<i>Achromobacter xylosoxidans</i> ssp. <i>denitrificans</i>	1	1		
<i>Acinetobacter baumannii</i>	6	5	1	
<i>Acinetobacter Iwoffii</i>	1	1		
<i>Acinetobacter</i> species	2	2		
<i>Aeromonas hydrophila</i>	1	1		
<i>Burkholderia cepacia</i>	1	0	1	
<i>Ralstonia pickettii</i>	1	1		
<i>Citrobacter amalonaticus</i>	1	1		
<i>Citrobacter braakii</i>	3	3		
<i>Citrobacter freundii</i>	8	8		
<i>Citrobacter koseri</i>	3	3		
<i>Edwardsiella hoshinae</i>	1	1		
<i>Edwardsiella tarda</i>	2	2		
<i>Enterobacter aerogenes</i>	10	10		
<i>Enterobacter cloacae</i>	13	11	2	
<i>Escherichia coli</i>	38	37	1	
<i>Ewingella americana</i>	1	1		
<i>Hafnia alvei</i>	3	3		
<i>Klebsiella oxytoca</i>	9	8	1	
<i>Klebsiella pneumoniae</i> ssp. <i>ozaenae</i>	1	1		
<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	26	25	1	
<i>Klebsiella pneumoniae</i> ssp. <i>rhinoscleromatis</i>	1	1		
<i>Kluyvera ascorbata</i>	1	1		
<i>Kluyvera cryocrescens</i>	1	1		
<i>Moellerella wisconsensis</i>	1	1		
<i>Morganella morgannii</i>	3	3		
<i>Ochrobactrum anthropi</i>	1	0	1	
<i>Pantoea agglomerans</i>	2	2		
<i>Proteus mirabilis</i>	5	5		
<i>Proteus vulgaris</i>	2	2		
<i>Providencia alcalifaciens</i>	2	2		
<i>Providencia rettgeri</i>	1	1		
<i>Providencia stuartii</i>	2	1		1
<i>Pseudomonas aeruginosa</i>	19	19		
<i>Pseudomonas oryzihabitans</i>	1	1		
<i>Salmonella</i> species	3	3		
<i>Serratia liquefaciens</i>	2	2		
<i>Serratia marcescens</i>	9	9		
<i>Serratia odorifera</i> 1	1	1		
<i>Serratia odorifera</i> 2	1	1		
<i>Serratia plymuthica</i>	1	1		
<i>Shigella sonnei</i>	2	2		
<i>Stenotrophomonas maltophilia</i>	3	3		
<i>Vibrio cholerae</i>	1	1		
<i>Yersinia enterocolitica</i>	1	1		
<i>Yokenella regensburgei</i>	1	1		
<b>Total</b>	<b>199</b>	<b>190</b>	<b>8</b>	<b>1</b>

Table 7. Breakdown of Organisms Tested by Class

Organism Class	Total	Genus			Species		
		Correct	Incorrect	No ID	Correct	Incorrect	No ID
Enterobacteriaceae	500	496 (99.2%)	3 (0.6)	1 (0.2)	490 (98%)	9 (1.8)	1 (0.2)
Nonfermentors	221	214 (96.8%)	4 (1.8)	3 (1.4)	208 (94.1%)	10 (4.5)	3 (1.4)
Oxidase Positive Fermentors	20	20 (100%)	0 (0)	0 (0)	20 (100%)	0 (0)	0 (0)

**CONCLUSION**

The BD Phoenix™ Automated Microbiology System:

- Provided rapid, accurate identification of recently isolated gram-negative bacilli.
- Provided rapid, accurate identification of stock isolates including gram-negative bacilli.
- Provided rapid, accurate identification of resistant gram-negative bacilli.