

Comparison of the Vitek Legacy, Vitek 2 Colorimetric AND Phoenix Systems for Identification of Fermenting and Non-Fermenting Bacteria of Clinical Origin

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REVISED ABSTRACT

Three commercial identification systems [Vitek Legacy (VK1) and Vitek 2 Colorimetric (VK2), bioMerieux, Hazelwood, MO; and Phoenix (PX), BD Diagnostics, Sparks, MD] were evaluated using 324 fresh clinical isolates and 93 frozen stock isolates of fermenting and non-fermenting gram-negative bacilli (GNB). A total of 287 fermenting GNB representing 35 species and 130 non-fermenting GNB representing 22 species were included in the comparison. No more than 25 isolates were tested for any given species. The identification was considered correct if all three methods gave the same species ID. Discrepancies were resolved by performing the API 20E plus additional conventional biochemical tests for the fermenting species and by performing reference biochemical identification for the non-fermenting species. Overall performance was as follows:

INTRODUCTION

The Phoenix™ Automated Microbiology System (PX) includes an instrument, software, disposable panels, broths for ID and AST, and an AST indicator. The disposable test panels contain 136 microdilution wells and are available in ID, ID/AST and AST only formats. The ID method employs modified conventional, fluorogenic, and chromogenic substrates. The NID (negative identification) panels were used in this study. Each NID panel contains 45 substrates including 16 fluorogenic, 14 fermentation, 8 carbon source, 5 chromogenic, and 2 miscellaneous substrates (urea and ornithine) plus two fluorescent positive controls. The panels are read at 20-minute intervals by the instrument. Final results are available in 2-12 hours for ID. The NID database contains 160 taxa, including 79 Enterobacteriaceae, 54 NFB and 27 miscellaneous species.

The Vitek 2 Colorimetric Automated Microbiology System (VK2) includes an instrument, software, and disposable cards. The ID method employs a 64-well plastic card that contains 47 modified conventional, and chromogenic substrates. Colorimetric measurements are taken every 15 minutes for a maximum incubation period of 10 h. The gram-negative database contains 142 taxa including 67 Enterobacteriaceae, 68 Non-Enterobacteriaceae and 7 other highly pathogenic organisms.

The purpose of this study was to compare the PX, VK2 and VITEK (legacy) (VK1) system for identification of clinical isolates of gram-negative rods (GNR).

FERMENTERS	Vitek Legacy	Vitek 2	Phoenix
No. Tested: 288	No. (%)	No. (%)	No. (%)
Correct to species without addl. tests	194 (67.4)	263 (91.7)	269 (93.8)
Correct to Genus or Group	37 (12.8)	6 (2.1)	3 (1)
Correct to species only after addl tests	54 (18.8)	12 (4.2)	2 (0.7)
Questionable ID or No ID given	3 (1)	3 (1)	0
Incorrect Genus or species	0	3 (1)	13 (4.5)
NON-FERMENTERS			
No. Tested: 129	No. (%)	No. (%)	No. (%)
Correct to species without addl. tests	115 (89.1)	123 (95.3)	108 (83.7)
Correct to Genus or Group	0	0	14 (10.9)
Correct to species only after addl tests	6 (4.7)	0	0
Questionable ID or No ID given	6 (4.7)	5 (3.9)	2 (1.6)
Incorrect Genus or species	2 (1.6)	1 (0.8)	5 (3.9)
OVERALL			
Correct to Genus/Species w/o addl tests	83%	94%	94.5%
Correct to Genus/Species after addl tests	97.4%	96.9%	95%

Without additional testing the Vitek 2 and Phoenix systems gave the highest number of correct identifications. After additional testing was performed all three systems provided correct identifications of >95%. The lowest misidentification rates were observed with the Vitek legacy and Vitek 2 systems.

METHODS

Strains, culture conditions. 324 fresh clinical isolates and 93 frozen stock isolates of fermenting and non-fermenting gram-negative bacilli (GNB) were used in this study. A total of 288 fermenting GNB representing 35 species and 129 non-fermenting GNB representing 21 species were included in the comparison. No more than 25 isolates were tested for any given species. Clinical isolates of GNB were taken from primary isolation plates (BAP or MacConkey Agar) and set up in our routine clinical microbiology laboratory on the VK1. The BAP purity plate from the VK1, which was incubated overnight at 35°C, was used as the source of the inocula for the VK2 and PX. Frozen (-70°C) stock isolates of previously characterized clinical strains were subcultured to BAPs for two consecutive days and then used as a source of inocula for parallel testing on all three systems.

Discrepancy Testing of organism identifications. When the answer given by all three instruments agreed at the genus and species level then the ID was considered to be correct. If one or more systems disagreed then testing was repeated in parallel on all three instruments. The results of the repeat tests were used for comparison purposes. If agreement was not unanimous upon repeat testing additional biochemical testing was performed as follows:

(i) For fermenting GNR an API 20E plus selected conventional biochemical tests were inoculated, (ii) for non-fermenting gram-negative rods (NFGNR) a system of 23 biochemical tests were employed as described by Schreckenberger (*Practical Approach to the Identification of Glucose Nonfermenting Gram-Negative Bacilli – A guide to Identification*, 3rd ed. Loyola University Chicago, 2005). In a few instances sequencing of 16S rRNA genes was performed using MicroSeq (ARUP Laboratories, Salt Lake City, UT).

Quality Control. Quality control strains were tested in all systems as recommended by each manufacturer.

Instruments:

- VITEK legacy with software WSVTK-R07.02
- VITEK 2 with software WSVT2-IDCT01
- Phoenix with software V3.34A/V3.54A

RESULTS

Table 1. Organisms Tested

Fermenters – 35 species	No.	Nonfermenters – 21 species	No.
<i>Aeromonas caviae</i>	7	<i>Achromobacter xylosoxidans</i>	11
<i>Aeromonas hydrophila</i>	3	<i>Acinetobacter baumannii</i>	24
<i>Aeromonas veronii biovar sobria</i>	1	<i>Acinetobacter haemolyticus</i>	1
<i>Citrobacter amalonaticus</i>	5	<i>Alcaligenes faecalis</i>	5
<i>Citrobacter braakii</i>	8	<i>Bordetella bronchiseptica</i>	1
<i>Citrobacter farmeri</i>	2	<i>Burkholderia cepacia</i>	3
<i>Citrobacter freundii</i>	25	<i>Chryseobacterium indologenes/gleum</i>	3
<i>Citrobacter freundii/youngae</i>	1	<i>Chryseobacterium meningosepticum</i>	10
<i>Citrobacter koseri</i>	25	<i>Cupriavidus pauculus</i>	1
<i>Citrobacter youngae</i>	4	<i>Delftia acidovorans</i>	2
<i>Edwardsiella tarda</i>	1	<i>Ochrobactrum anthropi</i>	2
<i>Enterobacter aerogenes</i>	16	<i>Pseudomonas aeruginosa</i>	23
<i>Enterobacter cloacae</i>	26	<i>Pseudomonas fluorescens</i>	3
<i>Enterobacter gergoviae</i>	1	<i>Pseudomonas luteola</i>	4
<i>Enterobacter sakazakii</i>	1	<i>Pseudomonas oryzihabitans</i>	4
<i>Escherichia coli</i>	25	<i>Pseudomonas putida</i>	2
<i>Hafnia alvei</i>	2	<i>Pseudomonas stutzeri</i>	5
<i>Klebsiella oxytoca</i>	17	<i>Pseudomonas stutzeri/Vb-3</i>	2
<i>Klebsiella pneumoniae</i>	23	<i>Ralstonia pickettii</i>	1
<i>Leclercia adecarboxylata</i>	1	<i>Rhizobium radiobacter</i>	4
<i>Morganella morganii</i>	14	<i>Stenotrophomonas maltophilia</i>	18
<i>Pantoea agglomerans</i>	2		
<i>Pasteurella multocida</i>	1		
<i>Plesiomonas shigelloides</i>	1		
<i>Proteus mirabilis</i>	25		
<i>Proteus penneri</i>	2		
<i>Providencia rettgeri</i>	2		
<i>Providencia stuartii</i>	11		
<i>Salmonella arizonae</i>	2		
<i>Salmonella</i> species	3		
<i>Serratia liquefaciens</i>	1		
<i>Serratia marcescens</i>	26		
<i>Serratia odorifera</i>	1		
<i>Shigella dysenteriae</i>	1		
<i>Shigella flexneri</i>	2		
Total	288	Total	129

Table 2. Accuracy of Identification on Three Systems

FERMENTERS	VITEK Legacy	VITEK 2	Phoenix
No. Tested: 288	No. (%)	No. (%)	No. (%)
Correct to species without addl. tests	194 (67.4)	263 (91.7)	269 (93.8)
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OVERALL			
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RESULTS

The organisms included in this study are shown in Table 1. The interpretations provided by the instrument software were used to place the identifications into five result categories as follows: (i) correct ID to species (unambiguous correct ID to the species level), (ii) Correct to Genus or Group (either ID to the genus level or low level of discrimination between two or more species in the same genus, including the correct species), (iii) Correct ID to species only after additional off line tests performed, (iv) no identification or questionable ID, and (v) misidentification (unambiguous incorrect ID to genus or species). Results were tabulated separately for Fermenters, Non-Fermenters and All organisms combined as shown in Table 2. Discrepant organism identifications are detailed in Table 3.

DISCUSSION

The PX and VK2 performed best overall without additional testing, 94.5% and 94% respectively. The lower percent of correct ID with VK1 was due to two factors. One was the requirement to perform simple off-line tests to separate certain species. Most of these (40/60) were either *K. pneumoniae* or *K. oxytoca* that required spot indole for speciation. The second factor was that VK1 does not attempt to separate the *C. freundii* complex. This accounted for 29/36 organisms identified to group level only. The largest number of misidentifications occurred with PX, 4.5% with fermenters and 3.9% with NFB. Most of these were due to incorrect species calls within the correct genus. All three instruments performed well with little or no maintenance or instrument failure.

Table 3. Organisms Giving Discrepant Calls or no ID on One or More Instruments

Reference ID	Phoenix ID	VITEK Legacy	VITEK 2
Non-Fermenting GNB			
<i>Acinetobacter haemolyticus</i>	<i>A. baumannii</i> complex	<i>A. baumannii</i> complex	<i>Acinetobacter haemolyticus</i>
<i>Alcaligenes faecalis</i>	<i>Achromobacter</i> sp.	<i>A. faecalis</i>	<i>A. faecalis</i> subsp. <i>faecalis</i>
<i>Bordetella bronchiseptica</i>	NO ID	<i>B. bronchiseptica</i>	<i>B. bronchiseptica</i>
<i>Burkholderia cepacia</i>	<i>P. putida</i>	<i>B. cepacia</i>	<i>B. cepacia</i>
<i>Cupriavidus pauculus</i> (<i>Ralstonia paucula</i>)	<i>Ralstonia paucula</i>	<i>B. bronchiseptica</i>	<i>Ralstonia paucula</i>
<i>P. aeruginosa</i>	<i>P. putida</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>P. putida</i>
<i>P. stutzeri</i>	NO ID	Low discrimination <i>P. stutzeri</i>	<i>P. stutzeri</i>
<i>P. stutzeri</i> – Vb-3	<i>P. putida</i>	<i>P. stutzeri</i>	<i>P. stutzeri</i>
Fermenting GNB			
<i>Citrobacter braakii</i>	<i>C. freundii</i> (3), <i>C. farmeri</i> (1)	<i>C. braakii</i> (4)	<i>C. braakii</i> (4)
<i>Citobacter braakii/farmeri</i>	<i>C. braakii</i>	<i>C. freundii</i> complex	<i>C. freundii</i>
<i>Citrobacter freundii</i>	<i>E. vulneris</i>	<i>C. freundii</i> complex	<i>C. freundii</i>
<i>Citrobacter freundii</i>	<i>C. freundii</i>	NO ID	<i>C. freundii</i>
<i>Citrobacter youngae</i>	<i>C. werkmanii</i>	<i>C. freundii/youngae</i>	<i>C. youngae</i>
<i>Enterobacter cloacae</i>	<i>E. intermedium</i>	<i>E. cloacae</i>	<i>E. cloacae</i>
<i>Enterobacter cloacae</i> (motility +)	<i>E. asburiae</i>	<i>E. cloacae</i> (mot +) <i>E. asburiae</i> (mot -)	<i>E. asburiae</i>
<i>Klebsiella oxytoca</i>	<i>K. pneumoniae</i>	<i>K. pneumoloxytoca</i>	<i>K. oxytoca</i>
<i>Klebsiella oxytoca</i>	<i>K. pneumoniae</i>	<i>K. pneumoloxytoca</i>	<i>K. oxytoca</i>
<i>Klebsiella oxytoca</i>	<i>K. oxytoca</i>	<i>K. pneumoloxytoca</i>	<i>K. ornithinolytica</i>
<i>Klebsiella pneumoniae</i>	<i>K. oxytoca</i>	<i>K. pneumoloxytoca</i>	Low discrimination <i>K. ornithinolytica</i> <i>K. pneumoniae</i>
<i>Serratia marcescens</i>	<i>S. plymuthica</i> (2)	<i>S. marcescens</i>	<i>S. marcescens</i>
Incorrect or No ID	20	3	4

CONCLUSION

- The PX and VK2 are acceptable new instruments for Identification of both fermenting and non-fermenting GNB likely to be recovered from human clinical specimens.

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