

Identification and Susceptibility Testing of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by Direct Inoculation from Positive BACTEC Blood Culture Bottles into Vitek 2

Marjan J. Bruins,* Peter Bloembergen, Gijs J. H. M. Ruijs, and Maurice J. H. M. Wolfhagen

Laboratory of Clinical Microbiology and Infectious Diseases, Isala Klinieken, 8021 AM Zwolle, The Netherlands

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Inoculation of an automated system for rapid identification (ID) and antimicrobial susceptibility testing (AST) directly from positive blood culture bottles will reduce the turnaround time of laboratory diagnosis of septicemic patients, which benefits clinical outcome and decreases patient costs. Direct test results, however, must always be confirmed by testing a pure overnight culture, which is the “gold standard.” We studied the accuracy of direct testing versus repeat testing in order to investigate the possibility of refraining from repeat testing. We also assessed the clinical risk of reporting results based on direct testing only. We inoculated Vitek 2 (bioMérieux) directly from 410 positive BACTEC 9240 (BD) blood culture bottles containing gram-negative rods and studied the ID and AST results. In a comparison of direct inoculation with the standard method, a total of 344 isolates of *Enterobacteriaceae* and *Pseudomonas aeruginosa* were tested, and 93.0% were correctly identified. Of the 39 (10.2%) samples that contained bacilli not identifiable by Vitek 2, only 1 gave a conclusive, correct result. The overall MIC agreement among 312 isolates was 99.2%, with 0.8% very major and 0.02% major error rates. Of only three (polymicrobial) samples, the direct susceptibility pattern would be reported to the clinician as too sensitive. Vitek 2 results obtained from direct inoculation of blood culture bottles containing gram-negative bacilli are safe enough for immediate reporting, provided that ID and AST are consistent. Repeat testing is not necessary, unless Gram stain or overnight subculture results raise doubt about the purity of the culture.

Shortening the turnaround time of microbiological analyses with an automated system for rapid identification and susceptibility testing of bacteria leads to a significant reduction of patient morbidity, mortality, and cost (1, 2). In particular, for patients with septicemia, rapid laboratory results are essential for appropriate treatment and improving clinical outcome (21). An automated blood culture system that monitors culture bottles for microbial growth minimizes the time necessary to detect positive blood cultures. Another way to save time might be to inoculate an automated system for rapid identification and susceptibility testing directly from positive blood culture bottles. The conventional way is to wait for the overnight subculture on agar to prepare a standard inoculum according to the manufacturer's guidelines.

Of all bacteria involved in bloodstream infections, the fast-growing *Enterobacteriaceae* will probably produce the quickest and best-correlating results for direct and standard inoculation. Several studies have compared direct and standard methods for different (combinations of) automated systems, but to our knowledge no study has yet done this for Vitek 2 (7, 8, 18, 20, 22).

We routinely inoculate Vitek 2 directly from BACTEC 9240 blood culture bottles that are positive for gram-negative rods. To check the direct results, we repeat the tests the next day with suspensions made from pure cultures according to the Vitek 2 manufacturer's instructions.

The two aspects we studied were (i) the accuracy of direct testing versus standard (repeat) testing in order to be able to refrain from repeat testing and (ii) the clinical risk of an approach using results of direct testing only.

MATERIALS AND METHODS

Samples. The Laboratory of Clinical Microbiology and Infectious Diseases of the Isala Klinieken, a 1,100-bed, multisite hospital in Zwolle, The Netherlands, processes ~9,500 blood cultures yearly by using the BACTEC 9240 system (software version V4.40A; culture bottles PLUS Aerobic/F and PLUS Anaerobic/F [BD, Erembodegem-Aalst, Belgium]). The data were collected from November 2000 to March 2003; during this period, every blood culture positive for gram-negative bacilli was inoculated into Vitek 2 directly, preferably from the aerobic bottle. Per patient, every first positive culture of a septicemic episode containing gram-negative rods that appeared to be monomicrobial on Gram staining was included in the study.

VT2. The Vitek 2 system (Vitek 2 software, version R02.03; Advanced Expert System [AES] software, version R02.00N [bioMérieux, Marcy l'Étoile, France]) was used with the ID-GNB card and the AST-N020 card for identification (ID) and antimicrobial susceptibility testing (AST), respectively, of gram-negative bacilli. The antimicrobial agents tested were amikacin, amoxicillin-clavulanic acid, ampicillin, cefalotin, cefepime, cefotaxime, cefoxitin, cefpodoxime, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, meropenem, nitrofurantoin, norfloxacin, ofloxacin, piperacillin, piperacillin-tazobactam, tobramycin, and trimethoprim-sulfamethoxazole.

Vitek 2 identifies gram-negative rods within 3 h at the species level by interpreting 41 fluorescent biochemical tests. If the biopattern does not match one particular taxon in the database, results are reported as “low discrimination” (2 to 3 taxa), “inconclusive” (>3 taxa), or “unidentified” (no match). Ambiguous results due to slowly metabolizing nonfermenters are reported as “various nonfermenting gram-negative bacilli.”

Susceptibility testing takes 5 to 19 h to obtain complete results. Vitek 2 needs a conclusive ID to establish and report definitive results. The AES analyzes whether ID and susceptibility data are consistent by consulting the phenotype descriptions in the database and, if necessary, recommends a correction in a MIC to complete the biological validation. The AES may also suggest a change in an

* Corresponding author. Mailing address: Isala Klinieken, Laboratory of Clinical Microbiology and Infectious Diseases, Rhijnvis Feithlaan 62, 8021 AM Zwolle, The Netherlands. Phone: 31-38-424-3111. Fax: 31-38-424-3146. E-mail: m.j.bruins@isala.nl.

interpretation from sensitive (S) to intermediate (I) or resistant (R), based on recognized resistance mechanisms, to prevent therapeutic failure. These changes of interpretation are according to the software parameters defined by the user (19). For the tests in the present study, interpretations as recommended by the National Committee for Clinical Laboratory Standards (15) and the AES configuration parameters set “natural resistance,” “European hospitals,” “never more susceptible,” and “least risk” were used. The AES knowledge base contains phenotypes of most *Enterobacteriaceae* and of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia*, *Pasteurella multocida*, and *Stenotrophomonas maltophilia* (only trimethoprim-sulfamethoxazole). If a species other than these is identified, expert analysis cannot be completed. For this reason, if the standard inoculation method is used, in our laboratory we inoculate Vitek 2 according to the manufacturer’s instruction, but only with isolates that are suspected of being *Enterobacteriaceae* or *P. aeruginosa*, based on their appearance on (differential) agar media and biochemical reactions such as the oxidase test.

Direct method. A serum separator tube (BD Vacutainer Systems, Plymouth, United Kingdom) was aseptically inoculated with 4 ml from a positive BACTEC 9240 bottle by using a 5-ml syringe, and a Gram stain was done. If the culture contained gram-negative rods and appeared to be monomicrobial, the tube was centrifuged at $1,525 \times g$ for 10 min, and the supernatant was carefully aspirated. With a cotton swab, the film of bacteria on top of the gel layer was removed and used to make a 0.5 to 0.63 McFarland suspension in 3 ml of 0.45% saline (modification of Waites et al. [22]). The suspension was processed according to the standard Vitek 2 inoculation procedure.

Standard method. Some of the blood culture fluid was inoculated onto a combination of agar plates, suited for culturing aerobic, anaerobic, and fastidious microorganisms. After overnight incubation, several colonies of isolates suspected of being *Enterobacteriaceae* or *P. aeruginosa* were used to make a 0.5 to 0.63 McFarland suspension in 0.45% saline. The suspension was processed according to standard Vitek 2 inoculation procedure.

Quality control. Weekly ID-GNB and AST-N020 cards were tested once each with *Klebsiella oxytoca* strain ATCC 700324 and *Escherichia coli* strain ATCC 25922, respectively.

Data analysis. Direct and standard inoculation were compared for *Enterobacteriaceae* and *P. aeruginosa*, except for AST of *Pantoea* spp., for which genus the AES does not contain expert data.

The Vitek 2 standard method results were used as the gold standard for ID testing, except in a few cases where the API system was necessary to provide the definitive identification. The Vitek 2 identifications *Klebsiella pneumoniae* subsp. *pneumoniae* (*planticolal/terrigena*) and *K. pneumoniae* subsp. *ozaenae* were considered identical and reported as *K. pneumoniae*; *K. oxytoca* and *K. oxytoca* (*planticolal/terrigena*) were reported as *K. oxytoca*. Our category “not identified” includes the Vitek 2 reports “inconclusive,” “unidentified,” and “terminated.”

For susceptibility testing, we compared direct and standard inoculation only of isolates with results biologically validated by Vitek 2 for both methods. The standard method results were considered the gold standard.

The MIC agreement (direct MIC within one twofold dilution of the standard MIC) (14) without any changes made by the AES and discrepancies in interpretation after AES analysis were determined for each antibiotic. A minor error was defined as a susceptibility result of “I” by the direct method and “S” or “R” by the standard method or “I” by the standard method and “S” or “R” by the direct method. A major error was defined as “R” by the direct method and “S” by the standard method, and a very major error was defined as “S” by the direct method and “R” by the standard method.

We also assessed the outcome of direct testing of blood cultures containing anaerobic, fastidious, and other gram-negative bacilli not identifiable by Vitek 2. We investigated whether these results could lead to misleading reports if reporting was based on direct testing only. For these isolates standard diagnostic methods such as the API system (bioMérieux) were used for identification.

If the agar plate culture of an apparently monomicrobial sample yielded two or more different species, we evaluated the therapeutic risk involved in the immediate reporting of the direct (mixed) susceptibility pattern.

RESULTS

Of the 410 blood cultures that met the study inclusion criteria, results of direct inoculation of six samples could not be retrieved because of missing or deleted data ($n = 2$), omission of direct testing ($n = 1$), or Vitek 2 malfunction ($n = 1$). Twenty-one samples (5.1%) that appeared to be monomicrobial in the Gram stain yielded more than one isolate in the

blood bottle’s subculture. Table 1 lists the results of 383 direct identifications from monomicrobial blood cultures. In all, 39 (10.2%) samples contained anaerobic, fastidious, or gram-positive bacilli or nonfermenting gram-negative rods other than *P. aeruginosa*. Of these, only one strain of *S. maltophilia* gave a conclusive result. Of 311 *Enterobacteriaceae* and 33 *P. aeruginosa* strains, 93.0% (320 of 344) were correctly identified, 2.6% (9 of 344) were reported as “low discrimination,” 2.3% (8 of 344) were not identified, and 2.0% (7 of 344) were misidentified according to our definitions. The five most frequently isolated species were *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae*.

Of the 320 correctly identified isolates of *Enterobacteriaceae* and *P. aeruginosa*, 8 were excluded from AST evaluation due to termination or omission of the standard method AST ($n = 4$), a standard method identification result of “low discrimination” ($n = 2$), an inconsistent direct ID and susceptibility pattern combination ($n = 1$), or identification as *Pantoea* spp. (no expert analysis possible) ($n = 1$). In all, 6,477 MICs were determined for 312 isolates by both methods. MIC agreement and interpretation discrepancies are shown in Table 2. The overall MIC agreement between direct and standard inoculation was 99.2%. Except for piperacillin (95.2%), all individual antimicrobial agents scored as $\geq 98.7\%$.

Most minor discrepancies occurred with cefalotin, nitrofurantoin, piperacillin, and cefuroxime. The overall minor error rate was 2.1% (133 of 6,477). Only one major error occurred for *E. coli* tested against ampicillin, which yielded an overall major error rate of 0.02% (1 of 6,477). Six very major errors occurred, which yielded an overall very major error rate of 0.1% (6 of 6,477). Murray et al. recommend calculating major and very major rates by dividing the number of discrepancies by the number of strains tested as sensitive and resistant, respectively, with the reference method (12, 13). According to this calculation, the major error rate would be 0.02% (1 of 5,508) and the very major error rate would be 0.8% (6 of 748). The very major errors were distributed among two isolates of *E. cloacae* and two isolates of *E. coli* and concerned test results associated with piperacillin, cefotaxime, and ceftazidime.

Of the 21 polymicrobial samples, 11 (52.4%) identifications yielded an unambiguous and conclusive result. For three of these samples, the AST results were reported as inconsistent; for one, they were terminated. Of the other seven samples with a falsely conclusive ID, three were reported with the susceptibility pattern of the most sensitive bacillus. One of these samples contained *K. oxytoca* and *P. aeruginosa*; the other two both contained two different phenotypes of *E. coli*.

DISCUSSION

In our study we focused on the direct inoculation of blood cultures containing gram-negative bacilli. Because in our experience direct inoculation of gram-positive cultures yielded unsatisfactory results, we discontinued this after a trial period (data not shown).

We used the Vitek 2 standard method as the gold standard for comparing direct and standard ID and AST results of Vitek 2 identifiable isolates, since its performance has been compared to reference methods extensively and with good results

TABLE 1. Results of direct inoculation of Vitek 2 ID-GNB card from blood cultures containing monomicrobial gram-negative rods

Organism group and name ^a	No. of strains				
	Tested	Correctly identified	Low discrimination	Not identified ^b	Misidentified (misidentification) ^d
Nonfermenters^c					
<i>Acinetobacter</i> spp.	4		1		3 (VNGNB)
<i>Alcaligenes</i> spp.	3			1	2 (VNGNB)
<i>Brevundimonas</i> spp.	1		1		
<i>Flavimonas</i> spp.	1		1		
<i>Ochrobactrum</i> spp.	1		1		
<i>Pseudomonas stutzeri</i>	1				1 (VNGNB)
<i>Ralstonia pickettii</i>	1				1 (VNGNB)
<i>Stenotrophomonas maltophilia</i>	3	1	1	1	
Anaerobic gram-negative rods					
<i>Bacteroides</i> spp.	14			14	
<i>Fusobacterium</i> spp.	2				2 (VNGNB)
Fastidious gram-negative rods					
<i>Haemophilus influenzae</i>	6		2	4	
<i>Streptobacillus moniliformis</i>	1				1 (VNGNB)
Gram-positive rods					
<i>Clostridium</i> spp.	1			1	
Enterobacteriaceae					
<i>Citrobacter koseri</i>	1	1			
<i>Citrobacter freundii</i>	3	2		1	
<i>Enterobacter aerogenes</i>	2	2			
<i>Enterobacter cloacae</i>	21	20		1	
<i>Enterobacter sakazakii</i>	1				1 (<i>Enterobacter cloacae</i>)
<i>Escherichia coli</i>	188	183	3		1 (VNGNB), 1 (<i>Klebsiella oxytoca</i>)
<i>Ewingella americana</i>	1		1		
<i>Klebsiella oxytoca</i>	13	13			
<i>Klebsiella pneumoniae</i>	30	26	1	3	
<i>Leclercia adecarboxylata</i>	1	1			
<i>Morganella morganii</i>	4	3			1 (<i>Bergeyella zoohelcum</i>)
<i>Pantoea</i> spp.	3	1	1	1	
<i>Proteus mirabilis</i>	25	25			
<i>Salmonella</i> spp.	7	6		1	
<i>Serratia marcescens</i>	11	11			
<i>Pseudomonas aeruginosa</i>	33	26	3	1	3 (VNGNB)
Total	383	321	16	29	17

^a Names determined by Vitek 2 standard method or API system.

^b Includes Vitek 2 reports "unidentified," "inconclusive," and "terminated."

^c Other than *Pseudomonas aeruginosa*.

^d VNGNB, various nonfermenting gram-negative bacilli.

(4, 5, 6, 10). In a few instances the API system helped out where the standard ID was inconclusive.

Routinely, we inoculate Vitek 2 only with isolates suspected of being *Enterobacteriaceae* or *P. aeruginosa* grown in pure culture from agar plates, for which the AES knowledge base contains the data to complete expert analysis of the AST. Although for ID testing Vitek 2 is approved by the Food and Drug Administration for a much larger range of gram-negative rods, in our experience and that of others its performance is best for identifying bacilli belonging to these groups (4, 6, 10). Therefore, direct and standard results of these species only (except *Pantoea* spp.) were compared; all other gram-negative bacilli were considered not identifiable by Vitek 2.

Direct inoculation of blood cultures containing gram-negative bacilli indistinguishable from organisms identifiable by Vitek 2 in the Gram stain might cause misleading reports.

However, in the present study, none of the isolated anaerobic, fastidious, or gram-positive bacilli or nonfermenters, other than *P. aeruginosa*, yielded an unambiguous Vitek 2 ID result, except for *S. maltophilia*, which was correctly identified.

With the direct method, 93.0% of *Enterobacteriaceae* and *P. aeruginosa* strains together and 94.5% of *Enterobacteriaceae* strains alone were correctly identified. For selecting a new system for identification, at least 90% overall agreement with a reference system and at least 95% accuracy in identifying commonly isolated organisms such as *Enterobacteriaceae* are recommended (13). We view our results as more than acceptable, since they are consistent with this and with the percentages determined in studies comparing Vitek 2 (used according to standard protocol) to reference methods (4, 6, 10, 16).

The top five most frequently isolated bacilli (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*) are

TABLE 2. MIC agreement and errors for antimicrobial agents between direct and standard method^a

Antimicrobial agent	No. of tests	MIC agreement			Error		
		MIC unagreement	MIC agreement	% MIC agreement	Minor	Major	Very major
Amikacin	302	0	302	100.0	1	0	0
Amoxicillin-clavulanic acid	311	4	307	98.7	4	0	0
Ampicillin	312	2	310	99.4	2	1	0
Cefalotin	311	3	308	99.0	15	0	0
Cefepime	301	0	301	100.0	0	0	0
Cefotaxime	310	4	306	98.7	5	0	2
Cefoxitin	301	0	301	100.0	4	0	0
Cefpodoxime	301	4	297	98.7	1	0	0
Ceftazidime	311	2	309	99.4	1	0	1
Cefuroxime	310	4	306	98.7	17	0	0
Cefuroxime axetil	300	4	296	98.7	24	0	0
Ciprofloxacin	311	0	311	100.0	0	0	0
Gentamicin	312	3	309	99.0	2	0	0
Meropenem	311	0	311	100.0	0	0	0
Nitrofurantoin	312	3	309	99.0	30	0	0
Norfloxacin	312	2	310	99.4	5	0	0
Ofloxacin	311	0	311	100.0	1	0	0
Piperacillin	312	15	297	95.2	17	0	3
Piperacillin-tazobactam	312	3	309	99.0	3	0	0
Tobramycin	302	0	302	100.0	1	0	0
Trimethoprim-sulfamethoxazole	312	0	312	100.0	0	0	0
Total	6,477	53 (0.8)	6,424 (99.2)		133 (2.1)	1	6

^a Values in parentheses are percentages.

similar to those reported from the United States and Canada and from Europe (3, 17).

The strains of *Enterobacteriaceae* or *P. aeruginosa* misidentified as *Bergeyella zoohelcum* and various nonfermenting gram-negative bacilli (no expert analysis possible) would be retested from a subculture before being reported. *Enterobacter sakazakii* identified as *E. cloacae* would not lead to inappropriate antimicrobial treatment. Probably a too-low inoculum was responsible for the misidentification of *E. coli* as *K. oxytoca*.

The overall 99.2% MIC agreement between direct and standard susceptibility testing was very high. For every antimicrobial agent the MIC agreement was >90%, as required by the selection criteria for an antimicrobial susceptibility testing system proposed by Jorgensen (9).

Overall, error rates were very low (major, 0.02%; very major, 0.8%). A too-low inoculum probably caused very major errors with direct testing of piperacillin, cefotaxime, and ceftazidime for only four isolates. The falsely sensitive results with cefotaxime and ceftazidime for *E. cloacae* would be of no consequence, since *Enterobacter* spp. capable of producing an inducible betalactamase would not be treated with any of these drugs.

Only 5.1% of the 410 samples inoculated directly into Vitek 2 were found to be polymicrobial when subcultured, which does not differ from other findings (11, 22). In seven samples there would have been a false report. Their identification and AST results were conclusive and consistent, so the fact that the cultures were not pure would not have been recognized. Of these, only three were reported with too-sensitive AST results, probably because of a lower inoculum or slower growth rate of the more resistant strain in the mixed culture.

We found that direct inoculation of Vitek 2 from BACTEC

9240 blood culture bottles to analyze gram-negative bacilli involved in bloodstream infection is safe enough for immediate reporting, provided that ID and AST are conclusive and consistent. Repeat testing by the standard method seems necessary only, if subculturing of the bottle yields isolates suspected of being *Enterobacteriaceae* or *P. aeruginosa* and the direct results are inconclusive. Nevertheless, direct inoculation should not be applied when the Gram stain raises any doubt about the purity of the culture.

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