East African Medical Journal Vol. 91 No. 4 April 2014
SUITABILITY OF VITEK 2 SYSTEM IN IDENTIFICATION AND SUSCEPTIBILITY TESTING OF GRAM NEGATIVE BACTEREMIAS BY DIRECT INOCULATION
D. Maina, MBChB, MMed Pathologist and E. Kagotho, MBChB, Department of Pathology, Aga Khan University hospital, P.O. Box 30270-00100, Nairobi, Kenya

Request for reprints to: Daniel Maina, Aga Khan University hospital, P.O. Box 30270-00100, Nairobi, email: daniel.maina@aku.edu

SUITABILITY OF VITEK 2 SYSTEM IN IDENTIFICATION AND SUSCEPTIBILITY TESTING OF GRAM NEGATIVE BACTEREMIAS BY DIRECT INOCULATION

D. MAINA and E. KAGOTHO

ABSTRACT

Objective: **To verify the accuracy of direct Vitek testing for blood cultures with Gram-negative bacilli.**
Design: **Validation study.**
Setting: **Aga Khan University Hospital Nairobi.**
Subjects: **Twenty two positive blood cultures.**
Main outcome measures: **Correct bacteria identification and errors for susceptibility testing.**
Results: **Of the 22 samples analysed 19(86%) were correctly identified by direct Vitek testing and three (14%) were unidentified. Of the three, one had mixed growth and the other two had pure growth on sub-cultures. Of the 19 cultures with antimicrobial susceptibility testing by direct Vitek, three had discrepancies for some antibiotics when compared with the conventional Vitek method. These discrepancies were minor errors that would not have had any clinical impact.**
Conclusion:**These data suggest that direct Vitek would provide acceptable identification and antimicrobial susceptibility testing results for Gram-negative bacilli. Compared to the standard method, the direct Vitek method would reduce turnaround time by at least twelve to twenty four hours.**

INTRODUCTION

Infection of the bloodstream remains a life-threatening occurrence. Blood cultures and their microbiological analysis are essential for the diagnosis of this infection (1,2). Rapid detection, identification, and antimicrobial susceptibility testing (AST) of bacteria from blood are crucial in patient management. Among the several methods currently used in clinical laboratories, culture is the most sensitive one for the detection of bacteria in blood samples. However, blood cultures require at least four to twenty four hours of incubation time and an additional twenty four to fourty eight hours for biochemical or immunological tests to identify bacteria and determine their susceptibility to antimicrobial agents (3).

The VITEK® 2 system (bioMérieux) is an automated bacteria identification and antimicrobial sensitivity testing platform that conventionally uses bacteria colonies obtained from cultures of clinical specimens. For blood this means sub-culturing of positive liquid cultures onto solid media from which distinct colonies can be processed further. This process of performing the sub-cultures first increases the turnaround time (TAT) for blood cultures. In order to decrease the TAT direct inoculation of positive blood cultures into Vitek (direct Vitek method) has been evaluated and found satisfactory for Gram negative bacilli (4-6). The Gram positives have had mixed results and the method has not been clinically adopted (7, 8). There are scanty data on direct inoculation of Gram negative cocci apart from *Acinetobacacter spp.* and no cocci were evaluated in our study.

There are several hospital microbiology laboratories in Nairobi that use Vitek but none has reported using the direct inoculation method for blood cultures.

This study aimed to verify the accuracy and hence the suitability of the direct Vitek method for the identification and susceptibility testing of Gram-negative bacilli directly from positive blood cultures in a local hospital laboratory.

MATERIALS AND METHODS

This was a method validation study carried out at the Aga khan university Hospital Nairobi, between July and September 2013.The protocol at this hospital recommends the use of both aerobic and anaerobic blood culture bottles in all cases except in pediatrics.

All blood cultures were screened using BACTEC™ 9050 (Becton, Dickinson and Company) and upon flagging a Gram stain was made from the culture bottle. Cultures that had a mono-microbial population of gram negative bacilli were processed through the direct Vitek method and the conventional procedure.

All positive blood cultures with gram negative bacilli were eligible for inclusion into the study. For any patient, only the first positive blood culture of any bacteraemic episode was included and cultures in which Gram stain indicated polymicrobial growth were excluded. A minimum of twenty specimens are required for method validation and twenty two blood cultures formed the study

Direct inoculation of the Vitek System: A serum separator tube was filled with four millilitres of aseptically aspirated blood from a positive blood culture bottle. It was then spun at 4000 rpm for ten minutes and the supernatant was removed. The buffy layer was emulsified into three millilitres of saline to obtain a McFarland standard of between 0.6 and 0.8.(Modified bruins method) (8).

Bacteria identification and AST were performed using the Vitek Gram negative card and AST-GN26 card respectively. The susceptibility results were categorised as susceptible, intermediate or resistant. Purity plating was also done to exclude mixed growth.

Conventional method of identification and susceptibility testing: At least two millilitres were aseptically aspirated from a positive blood culture bottle and plated onto blood agar, Sabouradsagar, chocolate agar and Mackonkey agar. These were then incubated for 16 hours. The distinct colonies were picked and emulsified in saline to obtain a McFarland of 0.5 and then put into Vitek for identification and susceptibility testing as above.

Comparison of the direct and conventional Vitek method: For both identification and susceptibility testing, the final report issued was based on the conventional method, which was the gold standard. All antimicrobial susceptibilies were interpreted using the clinical and laboratory standards Institute criteria (CLSI 2013).

A very major error was defined as a susceptible ('S') result by the direct method and resistant ('R') by the conventional method, and a major error was defined as 'R' by the direct method and 'S' by the conventional method. A minor error was defined as a susceptibility result of intermediate ('I') by the direct method and 'S' or 'R' by the conventional method, or 'I' by the conventional method and 'S' or 'R' by the direct method. Categorical agreement was recorded if the direct method and the conventional method were concordant.

RESULTS

A total of 22 blood cultures were analysed. The location of the patients at the time of draw is as shown (Table 1).

Table 1
Location of patients

Location	Frequency
CTICU	1
HDU	1
ICU	5
Medicine	2
NHDU	1
NICU	1
OPD	5
Pediatrics	3
Private ward	2
Surgery	1
Total	22

Identification: Of the twenty two blood cultures 19(86%) organisms were correctly identified by the direct Vitek method, and three (13.4%) were unidentified. Two of the unidentified were *Escherichia Coli*(*E. coli*) from anaerobic bottles and one was *Pseudomonas aeruginosa*. In one case of the unidentified *E. coli*, the purity plate had mixed growth. The other two cases had pure growth on purity plating. It's not obvious what the explanation is for the one pure *E. coli* that was unidentified but the lower rate recorded for *Pseudomonas*, a non-fermenter, may be partly a reflectionof the acknowledged superior performance of the Vitek 2 system inthe identification of *Enterobacteriaceae*, compared to non-fermenters,even from sub-cultures (9).

There was no case of misidentification (Table 2).

Table 2
Organismsidentified by direct Vitek testing

Organism	Tested	Correctly identified by direct Vitek	Unidentified by direct Vitek
Escherichia coli	9	7(78%)	2(22%)
Klebsiella pneumoniae	4	4(100%)	0 (0%)
Pseudomonas aeruginosa	2	1(50%)	1(50%)

Salmonella typhi	5	5(100%)	0 (0%)
Serratia marcescens	2	2(100%)	0 (0%)

Susceptibility testing: Of the 19 cultures with AST by direct Vitek, three had discrepancies for some antibiotics when compared with the conventional method. These were minor errors and none would have had any clinical impact. Tobramycin is rarely used in the country and piperacillin is in most instances used in combination with tazobactam (Table 3). No very major or major errors were noted.

Table 3
AST comparisons

Antibiotic	Total no of tests	No with categorical agreement	Minor errors
Amikacin	17	17(100%)	0 (0%)
Amoxicillin/clavulanate	17	17(100%)	0 (0%)
Ampicillin	18	18(100%)	0(0%)
Cefalotin	17	15(88.2%)	2(11.8%)
Cefepime	17	17(100%)	0(0%)
Cefotaxime	22	22(100%)	0(0%)
Cefpodoxime	17	17(100%)	0(0%)
Cefuroxime	17	17(100%)	0(0%)
Ceftazidime	17	17(100%)	0(0%)
Ciprofloxacin	22	22(100%)	0(0%)
Gentamicin	17	17(100%)	0(0%)
Meropenem	17	17(100%)	0(0%)
Piperacillin	17	16(94.1%)	1(5.9%)
Tobramycin	17	16(94.1%)	1(5.9%)
Trimethoprime/sulfamethoxazole	22	22(100%)	0(0%)
Total	271	267(98.5%)	4(1.5%)

DISCUSSION

In this study the direct Vitek testing gave the correct identification in 19(86%) of the cultures with only three unidentified, one of which had mixed growth. There was no case of misidentification. In the antimicrobial susceptibilities no very major or major errors were reported. There were only four instances of minor errors involving three antibiotics which would not have impacted negatively on patient care. These findings are similar to what has been reported elsewhere (5, 10).

In a South African study by Bramford *et.al,* 89% of Gram-negative bacilli were correctly identified to at least genus level by direct inoculation of the Vitek system from positive blood cultures. Higher rates were obtained for identification of *Enterobacteriaceae* (93%) than for non-fermenters (82%) (11).

Though the turnaround time was not explicitly documented in this study the direct Vitek results generally were twelve to twenty four hours earlier than with the conventional method.

After the causative pathogen is identified, streamlining to more-precise therapy of the shortest acceptable duration is implemented. In this way, the risks of death, morbid complications, increased duration of hospital stay (as a result of ineffective initial treatment), and emergence of resistance (due to extended treatment with broad-spectrum agents) are lowered (12).

Several approaches have been examined to reduce the time to microbial species identification in the diagnosis of Bloodstream infections (BSI) , including PCR-based methods and fluorescence *in situ* hybridisation for direct species identification of isolates from microscopically positive blood cultures (13, 14).

However these are not feasible in a clinical microbiology laboratory and the direct Vitek inoculation method remains a viable alternative for Gram-negative bacilli.

BSI due to Gram negative bacilli are more common than those due to Gram-positive organisms (contaminants excluded) in our hospital as compared

to hospitals in the west (unpublished data). This method of direct inoculation into Vitek is therefore relevant for us.

It is expected there would be some marginal increase in costs for those tests that are repeated for lack of identification. In these cases the TAT would match that of the conventional method since the purity plates would be available by the time a 'no identification' result is obtained.
The small number of isolates studied did not enable us to determine how the system performed between the aerobic and anaerobic blood cultures as in some instances there are wide differences in time to positivity between the two.

In conclusion, the direct Vitek method results were comparable to those by conventional method and may be adopted for clinical use in the laboratory. Compared to the standard method, the direct Vitek method would reduce turnaround time by at least twelve to twenty four hours.

REFERENCES

1. Bearman GM, Wenzel RP. Bacteremias: a leading cause of death. *Archives of medical research*. 2005;**36**:646-659.
2. Munson EL, Diekema DJ, Beekmann SE, Chapin KC, Doern GV. Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. *J. of Clin. Microbiol.* 2003;**41**:495-497.
3. Chen J-R, Lee S-Y, Yang B-H, Lu J-J. Rapid identification and susceptibility testing using the VITEK 2 system using culture fluids from positive BacT/ALERT blood cultures. *Journal of microbiology, immunology, and infection*= Wei mian yu gan ran za zhi. 2008;**41**:259.
4. Munoz-Dávila M, Yagüe G, Albert M, García-Lucas T. Comparative evaluation of Vitek 2 identification and susceptibility testing of Gram-negative rods directly and isolated from BacT/ALERT-positive blood culture bottles. *Euro J. of Clin. Microbiol & Infectious Diseases*. 2012;**31**:663-669.
5. Ng SY, Kwang LL, Tan TY. Identification of Gram-negative bacilli directly from positive blood culture vials. *J. of Med. Microbiol*. 2007;**56**:475-479.
6. Kerremans J, Goessens W, Verbrugh H, Vos M. Accuracy of identification and susceptibility results by direct inoculation of Vitek 2 cards from positive BACTEC cultures. *Euro. J. of Clin. Microbiol. and Infectious Diseases.* 2004;**23**:892-898.
7. Gherardi G, Angeletti S, Panitti M, Pompilio A, Di Bonaventura G, Crea F, *et al*. Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. *Diagn. Microbiol. and Infectious Disease.* 2012;**72**:20-31.
8. Lupetti A, Barnini S, Castagna B, Nibbering P, Campa M. Rapid identification and antimicrobial susceptibility testing of Gram-positive cocci in blood cultures by direct inoculation into the BD Phoenix system. *Clin. Microbiol. and Infection*. 2010;**16**:986-991.
9. Funke G, Monnet D, von Graevenitz A, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. *J. of Clin. Microbiol.* 1998;**36**:1948-1952.
10. Ling TK, Liu Z, Cheng AF. Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. *J. of Clin. Microbiol.* 2003;**41**:4705-4707.
11. Bamford C, Goodway J, Hoffmann R. Rapid identification and susceptibility testing of Gram-negative bacilli from blood cultures using the Vitek® 2 system. *Southern Afri. J. of Epidemiol. and Infection*. 2010;**25**:28-31.
12. Kollef MH. Broad-spectrum antimicrobials and the treatment of serious bacterial infections: getting it right up front. *Clin. Infectious Diseases*. 2008;**47**(Supplement 1):S3-S13.
13. Kempf VA, Trebesius K, Autenrieth IB. Fluorescent in situ hybridization allows rapid identification of microorganisms in blood cultures. *J. of Clin. Microbiol.* 2000;**38**:830-838.
14. Wellinghausen N, Wirths B, Essig A, Wassill L. Evaluation of the Hyplex BloodScreen multiplex PCR-enzyme-linked immunosorbent assay system for direct identification of gram-positive cocci and gram-negative bacilli from positive blood cultures. *J. of Clin. Microbiol.* 2004;**42**:3147-3152.