

Rapid Detection Tool for Vancomycin-Resistant *Enterococcus* (VRE) Directly from Human Specimens

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Abstract

A new, simple, and one-step tool for the direct detection of vancomycin-resistant *Enterococcus* (VRE) EPI-V[®] (Pilots Point LLC, Sarasota, FL) is presented. It contains all the ingredients in a unique stable powder form in a standard test tube. One only needs to add water, inoculate the specimen, and incubate. Specimens consisted of 553 sequential human rectal/perirectal swabs for VRE surveillance. The presence of VRE was denoted by the production of two sequential color changes corresponding to growth in bile-esculin and production of a positive PYR reaction. The EPI-V[®] tool was compared to reference VRE detection methods. EPI-V[®] showed a sensitivity of 102% and a specificity of 98.4% for the detection of VRE. The EPI-V[®] tool offers significant advantages: no skilled technologist time required, simple quality control, highly conserved incubator and refrigerator space, and low cost.

Keywords

VRE, Stool, Carriage, Detection

1. Introduction

Both asymptotically colonized and infected patients serve as reservoirs for transmission of VRE, as well as contaminated surfaces and patient care equipment. European Union and United States public health agencies have developed recommendations for preventing the transmission of vancomycin resistance within and among hospitals and nursing homes. Current surveillance methods require a number of sequential steps and need a special series of culture media and identification tests. A highly skilled and trained medical technologist is needed

to perform the analysis.

A new, simple, and one-step tool for the detection of VRE (as defined as a minimum inhibitory concentration (MIC) to vancomycin of 6 mcg/mL) directly from rectal/perirectal specimens was compared to a reference, or to predicate method (Food and Drug Administration (FDA)). The tool (EPI-V[®] Pilots Point LLC, Sarasota, FL) contains all the required ingredients in a stable powder form, in a standard test tube. The presence of VRE in the rectal swab is denoted by the production of a two sequential biochemical reactions inside the test tube: substrate hydrolysis representing bile-esculin followed by PYR substrate hydrolysis. Bile-esculin plus PYR is the generally accepted identification of the genus *Enterococcus* [1]-[13].

2. Material and Methods

Specimens consisted of 553 sequential human rectal/perirectal surveillance swabs obtained as part of the ongoing surveillance program. The collection device was a Culturette[™] II (Becton Dickinson and Company, Cockeysville, MD). There was no prior notification of individuals collecting the specimens. The Culturette[™] II swab was twirled vigorously in 1 mL of pH 7.0, 50 mM HEPES (Sigma-Aldrich, St. Louis, MO) buffer so that each of the two methods would be challenged with the same inoculum.

3. Standard Culture Method

The FDA predicate VRE culture method is Bile Esculin Azide agar with 6 mcg/mL vancomycin (BEAV) (Remel, Lenexa, KS) [http://www.accessdata.fda.gov/cdrh_docs/reviews/K091025.pdf]. From the HEPES buffer, 0.1 mL of the extracted patient specimen was plated on the surface of the BEAV agar plate. After incubation for 24 hours at 35°C in ambient air, colonies that were brown-black were gram stained. Colonies showing gram positive cocci in chains were then tested by the PYR reaction (Becton, Dickinson and Company, Cockeysville, MD). The MIC to vancomycin from PYR positive colonies was performed by the Etest[®] method (bioMérieux, Durham, NC).

4. EPI-V[®]

The EPI-V[®] tool is in a powder format, in a flange-capped test tube, and stored at room temperature. To use, 3 mL of sterile water was added to dissolve the powder. To this solution, 0.1 mL of the extracted patient's specimen was added. EPI-V[®] test tubes were incubated for a maximum of 24 hours at 35°C in ambient air to call a specimen negative. However, color development at any time, denoted a positive result. Test tubes demonstrating a brown-black color (bile-esculin positive) were tilted to coat the disk present in the EPI-V[®] cap. This cap contains an enhanced reagent for the detection of PYR hydrolysis [14]-[17]. The development of a bright fuchsia color within 15 seconds is a positive reaction for PYR. Positive bile-esculin and PYR results demonstrated the presence of VRE (see **Figure 1** and **Figure 2**).

In order to determine if a false-positive or false-negative reaction had occurred from the EPI-V[®], approximately 0.1 mL was subcultured from all tests tubes to the surface of a Bile Esculin Azide agar plate containing 6 mcg/mL vancomycin. Plates were incubated at 35°C for 24 hours in ambient air. All colonies compatible with *Enterococcus* were then identified to species. If an *Enterococcus* species was isolated, an Etest[®] MIC to vancomycin was performed. All isolates were identified to species using the Vitek II system (bioMérieux, Durham, NC).

5. Results

The EPI-V[®] tool, from a diluted specimen, demonstrated a sensitivity of 102% and a specificity of 98.4%. **Table 1** presents the comparison of the EPI-V[®] results versus the Bile Esculin Azide with 6 mcg/mL vancomycin (BEAV) culture method for 553 stool samples. **Table 2** presents the time course of the 250 positive analyses by the EPI-V[®] tool.

6. Discussion

The EPI-V[®] tool showed excellent sensitivity and specificity relative to the Bile Esculin Azide FDA referenced method and required only a maximum of 20 hours. In addition, the EPI-V[®] tool offered several significant ad-



Figure 1. Negative EPI-V[®]. Negative EPI-V[®] liquid is straw-colored and the disk in the cap is white.



Figure 2. Positive EPI-V[®]. Positive EPI-V[®] liquid is dark brown-black and the disk in the cap is bright fuchsia.

Table 1. Comparison of EPI-V[®] and Bile Esculin Azide media for the recovery of VRE from stool samples.

	BEAV positive	BEAV negative
EPI-V [®] positive	250	16
EPI-V [®] negative	7	280

Table 2. Time to positive of EPI-V[®] from 250 positive stool samples.

Time	Number of specimens positive	Cumulative number of specimens positive
5 h	22	22
6 h	26	48
7 h	15	63
8 h	21	84
9 h	33	117
10 h	17	134
16 h	83	217
18 h	18	235
20 h	15	250

vantages over the FDA referenced method. These included: no skilled technologist time required, simple quality control, highly conserved incubator and refrigerator space, and approximately 1/4 - 1/3 cost of agar based methods. The simplicity and ease of use of the EPI-V[®] epidemiology tool allows for more efficient and timely processing of large numbers of patient surveillance specimens for VRE. This new tool offers the clinical microbiologist, hospital epidemiologist, or infection control practitioner, flexibility as to the timing of collection and number of rectal or perirectal patient surveillance cultures.

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