Verification of Seegene’s Allplex Entero-DR Assay

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ABSTRACT

**Objectives:** Rapid detection of carbapenemase-producing organisms (CPO), extended-spectrum β-lactamases (ESBL), and vancomycin-resistant enterococcus (VRE) is important. Data suggest Nucleic Acid Amplification Tests (NAAT) are more sensitive than culture. We previously evaluated the Allplex Entero-DR NAAT assay (Seegene). CPO-testing was done previously on 200 well-characterized isolates, 53 rectal ESWabs, and 11 rectal swab on-target CPO-positive broth cultures. The purpose of this study was to complete verification of the assay's ability to detect CTX-M ESBLs and VRE (vanA and vanB).

**Methods:** ESBL and VRE testing was completed using: (A) 61 well-characterized isolates including 33 vanA, 6 vanB, and 22 CTX-M; (B) 36 rectal ESWabs including 14 CTX-M, 10 vanA, and 12 non-ESBL/VRE; and (C) 20 rectal swab on-target positive broth cultures (10 CTX-M and 10 vanA). (D) Limit of Detection (LOD) for VRE was calculated using 2 QC strains following manufacturer direct-from-specimen-protocols using 10E7-10E9 CFU/L concentrations with Xpert® vanA/vanB (Cepheid) (our laboratory's current method) as reference using colony counts to correct for actual concentration tested.

**Results:** For CPO-testing, all targets were detected and all non-targeted-CPO/non-CPO were negative for each type as previously presented. For ESBL and VRE testing, (A) of the 61 well-characterized isolates, all VRE and CTX-M were detected. VRE-detection sensitivities/specificities (Sn/Sp) were 100%; respective Sn/Sp 95%CI were vanA (87.61-100/85.7-100) and vanB (55.72-100/92.2-100) and CTX-M ESBL-detection Sn/Sp were 100% (Sn/Sp95%CI 82.45-100/89.32-100). (B) Of the 37 rectal ESWabs, all target vanA and CTX-M were detected and all non-target were not-detected. vanA-detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/84.76-100) and CTX-M detection Sn/Sp were 100% (Sn/Sp95%CI 74.85-100/82.45-100). (C) Of the 20 rectal swab on-target positive broth cultures, all vanA and CTX-M were detected. vanA-detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/67.91-100) and CTX-M detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/67.91-100). (D) LOD (cfu/L) are shown (Table).

**Conclusion:** The SeeGene Allplex Entero-DR Assay was highly accurate for CPO, VRE and CTX-M detection but has a lower vanB LOD compared to Cepheid.

INTRODUCTION

Rapid detection of carbapenemase-producing organisms (CPO), extended-spectrum β-lactamases (ESBL), and vancomycin-resistant enterococcus (VRE) is important. Data suggest Nucleic Acid Amplification Tests (NAAT) are more sensitive than culture. We evaluated the Allplex Entero-DR NAAT assay (Seegene). CPO-testing was done previously on 200 well-characterized isolates, 53 rectal ESWabs, and 11 rectal swab on-target CPO-positive broth cultures. The purpose of this study was to complete verification of the assay’s ability to detect CTX-M ESBLs and VRE (vanA and vanB).

METHOD

ESBL and VRE testing was completed using: (A) 61 well-characterized isolates including 33 vanA, 6 vanB, and 22 CTX-M; (B) 36 rectal ESWabs including 14 CTX-M, 10 vanA, and 12 non-ESBL/VRE; and (C) 20 rectal swab on-target positive broth cultures (10 CTX-M and 10 vanA). (D) Limit of Detection (LOD) for VRE was calculated using 2 QC strains following manufacturer direct-from-specimen-protocols using 10E7-10E9 CFU/L concentrations with Xpert® vanA/vanB (Cepheid) (our laboratory’s current method) as reference using colony counts to correct for actual concentration tested.

RESULTS

For CPO-testing, all targets were detected and all non-targeted-CPO/non-CPO were negative for each type as previously presented (AMMI/CACMID 2019).

For ESBL and VRE testing:

(A) Of the 61 well-characterized isolates:
- all VRE and CTX-M were detected.
- VRE-detection sensitivities/specificities (Sn/Sp) were 100%
- respective Sn/Sp 95%CI were vanA (87.61-100/85.7-100) and vanB (55.72-100/92.2-100) and CTX-M ESBL-detection Sn/Sp were 100% (Sn/Sp95%CI 82.45-100/89.32-100).

(B) Of the 37 rectal ESWabs:
- all target vanA and CTX-M were detected and all non-target were not-detected.
- vanA-detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/84.76-100) and CTX-M detection Sn/Sp were 100% (Sn/Sp95%CI 74.85-100/82.45-100).

(C) Of the 20 rectal swab on-target positive broth cultures:
- all vanA and CTX-M were detected. vanA-detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/67.91-100) and CTX-M detection Sn/Sp were 100% (Sn/Sp 95%CI 67.91-100/67.91-100).

(D) LOD (cfu/L) are shown (Table).

CONCLUSION

The SeeGene Allplex Entero-DR Assay was highly accurate for CPO (AMMI/CACMID 2019 data), VRE and CTX-M detection but has a lower vanB LOD compared to Cepheid.