ABSTRACT (Revised)

Background: Antiretroviral drug resistance testing is a standard of care in the management of individuals with HIV-1 infection. Currently, the Sanger sequencing-based ViroSeq™ antiretroviral drug resistance detection assay approved by FDA for clinical use in the U.S. We conducted a multicenter FDA registration trial to evaluate the performance characteristics of a new, next-generation sequencing assay, the Sentosa® SQ HIV Genotyping Assay (Sentosa; Abbott Molecular Systems, Inc.).

Methods: For limit of detection (LoD), an HIV-1 group M subtype B reference strain was tested in 20 replicates each at 500, 1,000, and 2,000 copies/mL, with Sentosa HIV, while group M subtypes A, C, D, F, G, H, J, and K (3 strains each) were tested at 1,000 copies/mL. For limit of quantitation (LoQ), 20 clinical plasma specimens containing known drug resistance mutations (DRM) occurring in the protease (PR), reverse transcriptase (RT), and integrase (INT) of HIV-1 reference strain 89GR, recovered from the plasma of infected individuals. It is the first commercial, semi-automated, sample-to-result, NGS assay designed for this purpose and is intended for use with the Sentosa® EXKIT® and SQ301® instruments. Sentosa HIV is specifically designed to interrogate 2 different regions of the HIV-1 genome: ~1,500 bp (PR and RT codons 1 to 99 and 1 to 337, respectively) and ~1,000 bp (INT codon 1 to 28).

RESULTS

• LoD (95% detection rate) of 1,000 copies/mL was established for HIV-1 group M and confirmed with subtypes A, C, D, F, G, H, J, and K.

• At 1,000 copies/mL, DRM present at frequencies ≥20% were detected in 90% of replicates, while DRM present at ≤5% were detected in <90% of replicates.

• LoQ (95% detection rate) of 500 copies/mL was confirmed with subtypes A, C, D, F, G, H, J, and K.

• Clinical sensitivity and specificity were 98.2% and 99.9%, 65% and 99.6%, and 96.1% and 99.7% for PR / RT, INT, and overall DRM detection, respectively, when DRM was detected at >20% frequency (for direct comparison to Sanger sequencing-based methods).

INTRODUCTION

The Sentosa® SQ HIV Genotyping Assay (Sentosa HIV) is a new, next-generation sequencing (NGS) assay intended for use in the detection of the HIV-1 group M drug resistance mutations (DRM) occurring in the protease (PR), reverse transcriptase (RT), and integrase (INT) of HIV-1 reference strain 89GR (previously determined by ViroSeq™ and a laboratory-developed Vela Integrase assay) were tested at 3 laboratory sites.

METHODS

LoD: HIV-1 group M subtype B reference strain was tested in 20 replicates each at 500, 1,000, and 2,000 copies/mL, with Sentosa HIV, while group M subtypes A, C, D, F, G, H, J, and K (3 strains each) were tested at 1,000 copies/mL. For limit of quantitation (LoQ), 20 clinical plasma specimens containing known DRM were tested in 3 laboratory sites.

Clinical Sensitivity / Specificity: The Sentosa® SQ HIV Genotyping Assay (Sentosa HIV) has been tested in multiple centers with high reproducible detection of genotypic antiretroviral drug resistance mutations among HIV-1 group M strains found in clinical plasma specimens.

RESULTS

• LoD (95% detection rate) of 1,000 copies/mL was established for HIV-1 group M and confirmed with subtypes A, C, D, F, G, H, J, and K.

• At 1,000 copies/mL, DRM present at frequencies ≥20% were detected in 90% of replicates, while DRM present at ≤5% were detected in <90% of replicates.

• LoQ (95% detection rate) of 500 copies/mL was confirmed with subtypes A, C, D, F, G, H, J, and K.

• Clinical sensitivity and specificity were 98.2% and 99.9%, 65% and 99.6%, and 96.1% and 99.7% for PR / RT, INT, and overall DRM detection, respectively, when DRM was detected at >20% frequency (for direct comparison to Sanger sequencing-based methods).

CONCLUSION

As a semi-automated, sample-to-answer, next-generation sequencing assay, Sentosa HIV provides sensitive and specific detection of genotypic antiretroviral drug resistance mutations among HIV-1 group M strains found in clinical plasma specimens.