Evaluation of the Abbott RealTime HIV-1 Assay

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Materials and Methods

Sample selection, preparation, and storage. Plasma samples previously submitted to ARUP for HIV PCR testing were randomly selected to represent both negative and positive samples. HIV-1 RNA Quantitative Real-Time PCR (cDNA) (Amplicor) assay was performed using the ABI 7500 Real-Time PCR system (Applied Biosystems). Positive controls were HIV-1 DNA samples collected, desiccated, and stored at -70°C before testing. One hundred seventy-one (171) samples were included in the study. Ninety-eight (98) 0.5 mL samples produced valid quantitative results with a mean inter-assay CV of 5.8%, both occurring at approximately 2 log10 copies/mL. The remaining 73 (73) samples could not be quantified due to invalid results, most likely due to the limit of detection of the assay, high inter-assay CVs, or interfering substances. Twenty (20) samples were negative, 51 (51) were positive, and 42 (42) were indeterminate. Among the 42 (42) indeterminate samples, 14 (14) samples were negative, 9 (9) had results 0.5 log10 copies/mL below the limit of detection (LOD), 5 (5) were positive with > 0.5 log10 copies/mL above the LOD, and 14 (14) samples were indeterminate due to assay failure.

Conclusions

1. The RealTime assay correlated well with Amplico, determining regression slopes of 0.971 and 0.970 copies/mL for the 1 mL and 0.5 mL protocols, respectively, and R2 values of 0.984 and 0.983 for the 1 mL and 0.5 mL protocols, respectively.

2. Given the small sample size, it was not possible to calculate a meaningful correlation coefficient for the 0.25 mL protocol.

3. The discrepancy between Amplico- and RealTime-derived results was not seen in the genotype panel samples.

4. The RealTime assay demonstrated broad, linear dynamic range of nearly 5 log10 copies/mL, (-6 to 0 copies/mL for the 1 mL and 1 to 6 log10 copies/mL for the 0.5 mL protocol) for the 0.5 mL protocol.

5. The limit of detection of the RealTime assay was determined by probit analysis to be 21.94 0.5 copies/mL, using HIV-1 DNA sample and 24.54 copies/mL, using HIV-1 RNA sample.

6. The RealTime assay was the only assay that reliably detected and quantitated Group D samples.

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