

Validating 16 Member Pooled APTIMA® HIV-1 RNA testing

<i>Abstract Category:</i>	Strategies for Routine Screening for Acute HIV-Infection
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PROJECT

The Primary HIV Infection (PHI) Study uses an Aptima® HIV-1 RNA pooled testing strategy on seronegative specimens to screen for PHI at the New York State Department of Health Laboratory at Wadsworth (NYSDOH) and the Florida Bureau of Laboratories (FBOL) in Jacksonville, Florida.

ISSUES

Report of negative Aptima® test results requires validation of pooled testing. The FDA requires a lower limit of detection of 5,000 copies/ml of HIV-1 RNA per screening pool, documentation of sensitivity based on pool size, and verification of no sample carryover or degradation during the pooling process. Reporting a positive Aptima® test result isolated from pooled testing is achieved by testing positive individual specimens in/ duplicate as directed by the product label.

RESULTS

Three low level HIV-1 RNA secondary standards were prepared by CDC from a World Health Organization (WHO) HIV-1 RNA Quantification Standard lot# 200207029 (cat. 3443) certified at 150,000 copies/ml spiked into 1.250 ml of HIV-1 negative plasma. The WHO Standard was diluted in negative plasma to the following calculated levels: 1070, 2130, and 5330 copies/ml using calibrated laboratory pipettes. Viral loads of the WHO secondary standards were each measured in triplicate in three runs; the means and standard deviations, based on analysis of variance, with rounding to two significant digits, were the following: 900 (SD 200), 2500 (SD 1300), and 6100 (SD 1200) copies/ml. CDC sent 25 vials of each standard (blinded) to NYSDOH and FBOL to be analyzed 24 times in 16-member pools with 15 known HIV-negative specimens. Reactive pools were tested in duplicate to verify that they were repeatedly reactive. All pooled standards were repeatedly reactive in all 24 analyses in both laboratories except one with an initial invalid result that retested reactive.

LESSONS LEARNED

Our estimated lower limit of detection (sensitivity) of the Aptima® HIV-1 RNA test in a 16 member pool of 900 (2 SD 500-1300) copies per ml met the FDA-required lower limit of detection for a screening pool. We plan to expand validation to larger pools for PHI screening for further cost savings.