Validation of the GEN-PROBE® APTIMA® HIV-1 RNA Qualitative Assay for use with Dried Blood Spots (DBS)

Timothy J. Sullivan[1], Katherine N. Nerses[2], Monica M. Parker[1]
[1]Wadsworth Center, New York State Department of Health, Albany, NY, [2]Emory University, Atlanta, GA

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Background

The Wadsworth Center performs Western blot testing on dried blood spots (DBS) submitted by community-based HIV rapid testing sites to confirm reactive HIV rapid tests. When antibodies do not confirm, there are no additional tests available to resolve the discordance. This issue has become particularly relevant as many sites have begun to use the Alere Determine™ HIV Ag/Ab rapid test which can detect early HIV-1 infections well before the Western blot will be positive. Therefore, we sought to validate the APTIMA® HIV-1 RNA Qualitative Assay for use with DBS specimens.

Methods

Limit of Detection (LOD) and Stability Studies

- EDTA-whole blood spiked with HIV-1 positive plasma (Acrometrix) to final conc. of 20,000, 10,000, 5000, 2500, 1000, and 500 HIV-1 RNA copies/ml.
- 50µl spotted onto Whatman 903 cards and dried at ~23°C O/N
- Stored with desiccant at -20°C, except,
  - DBS for stability analyses stored at either ~23°C or 37°C without desiccant.

Blinded Accuracy Studies

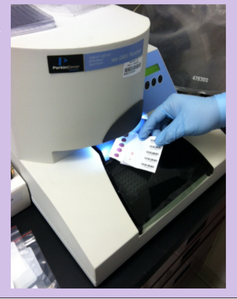
- DBS prepared as described from HIV-1 positive and negative blood specimens submitted for clinical testing and blinded.
- Paired plasma tested according to standard protocols.

Testing Procedure

- 2 X 6-mm punches (Figure) added to 530µl elution buffer (1mM EDTA, 1mM EGTA, 3% lithium lauryl sulfate in PBS); incubated at 4°C O/N.
- 500µl eluate tested on the APTIMA® HIV-1 RNA Qualitative Assay.

Figure

A



(A) Automated Wallac DBS puncher.
(B) Whatman 903™ Collection Card
- Two 6-mm punches shown

B

Results

Table 1. Limit of Detection (LOD)

HIV-1 RNA (copies/ml)	HIV-1 RNA (copies/reaction)	# Reactive/ # Tested	% Reactive
500	10	1/7	14.3
1000	20	2/7	28.6
2500	50	14/17*	82.4
5000	100	7/7	100
10000	200	4/4	100
20000	400	4/4	100

*A sample preparation error was suspected for 2 of the 2500 copies/mL DBS. For the reliable DBS at 2500 copies/mL, 14/15 (93.9) were positive.

Limit of Detection = 5000 copies/mL or 100 copies/reaction.

Table 2. Stability Study

Storage temp (21 days)	HIV-1 RNA (copies/ml)					
	500	1000	2500	5000	10000	20000
RT	NR	NR	R	R	R	R
37°C	R	NR	R	R	R	R

NR, Nonreactive; R, Reactive

DBS stored for up to 21 days at ≤ 37°C are suitable for qualitative RNA.

Table 3. Accuracy of APTIMA® using DBS

	APTIMA® Reactive	APTIMA® Nonreactive
HIV-1 Ab Positive	18	2*
HIV-1 Ab Negative	0	10

*Both specimens tested APTIMA® reactive on repeat analysis.

- **HIV-1 RNA was detected in 90% HIV-1 antibody-positive DBS.**
- **HIV-1 RNA was not detected in any HIV-1 negative DBS.**

- Additional testing: 2 false-negative DBS repeated: RNA detected in both.
- Comparable volume of paired plasma tested; RNA detected in 1 of the 2.
- Suggests that the amount of HIV-1 RNA in both DBS was near the LOD.

Case Study

Community-based Rapid Testing Site (Day 0)
- Determine HIV-1/2 Ag/Ab Combo = Ab positive
- DBS collected and sent to Wadsworth Center for confirmation

Wadsworth Center (Day 4)
- Western blot on DBS = Negative

Patient referred for testing at medical center (Day 4)
- Abbott ARCHITECT HIV Ag/Ab Combo = Reactive
  - S/CO = 31.26, 30.79, 30.31
- Bio-Rad Multispot = Indeterminate (Recombinant HIV-1 spot only)
- Serum sent to Wadsworth Center for APTIMA HIV-1 RNA

APTIMA HIV-1 RNA Assay at Wadsworth Center
- Serum (from Day 4) = RNA Detected (RLU = 1,162,847, S/CO = 21.99)
- DBS (from Day 0) = RNA Detected (RLU = 1,205,763, S/CO = 26.17)

Conclusions

- APTIMA® HIV-1 RNA Qualitative Assay detect HIV-1 RNA in DBS specimens with at least 5000 RNA copies/ml.
- Storing DBS at elevated temperatures for 21 days had no effect on the LOD for the assay.
- Testing of HIV-positive DBS prepared from clinical blood samples was 90% accurate.
- Testing DBS using the APTIMA assay is feasible, but the LOD relative to plasma and serum should be clearly noted when reporting negative results.

Works Cited

Kerr RJS, Player G, Fiscus SA, Nelson JAE. Qualitative human immunodeficiency virus RNA analysis of dried blood spots for diagnosis of infections in infants. *Journal of Clinical Microbiology*. 2009; 47(1): 220-22.