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Comparison of the Gen-Probe Aptima HIV-1 and Abbott HIV-1 Qualitative Assays with the Roche Amplicor HIV-1 DNA Assay for Early Infant Diagnosis Using Dried Blood Spots

Julie A. E. Nelson[1,2,*], **J. Tyler Hawkins**[1,2], **Maria Schanz**[1,2], **Katie Mollan**[1], **Melissa B. Miller**[3], **John L. Schmitz**[1,3], and **Susan A. Fiscus**[1,2,3]

[1]UNC Center for AIDS Research, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[2]Department of Microbiology and Immunology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[3]Department of Pathology and Laboratory Medicine, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Abstract

Background—The current gold standard for infant diagnosis of HIV-1 is the Roche Amplicor Qualitative DNA assay, but it is being phased out.

Objective—Compare the Abbott qualitative assay and the Gen-Probe Aptima assay to the gold standard Roche DNA assay using dried blood spots (DBS).

Study design—The Gen-Probe Aptima and Abbott qualitative HIV-1 assays were compared to the Roche DNA assay for early infant diagnosis. Specificity and sensitivity were determined for the three assays using DBS from 50 HIV-exposed uninfected infants and 269 HIV-1 infected adults from North Carolina, respectively. All of the negative and 151 of the positive DBS had valid results on the 3 different assays, and an additional 118 positive DBS had valid results on the Roche DNA and Aptima assays.

Results—All three assays were very specific. The Roche DNA assay was the most sensitive (96.7%) over a wide range of HIV PVL, including samples with PVL<400 copies/ml. Restricted to samples with PVL>400 copies/ml, the Gen-Probe Aptima assay had sensitivity (96.5%)

*Corresponding author: Julie A. E. Nelson, Center for AIDS Research, CB#7295, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295.

Competing interests

Abbott Qualitative HIV-1 Assay kits were provided by Abbott Molecular. JAEN and SAF have served as advisory board members for Roche Diagnostics and Hologic Gen-Probe, and have served as speakers for Abbott Molecular.

Ethical approval

None required.

comparable to the Roche DNA assay (98.8%). The Abbott Qualitative assay was the least sensitive and only had sensitivity above 95% among samples with PVL over 1000 copies/ml.

Conclusions—The Abbott HIV-1 Qualitative assay was not as sensitive as the comparator assays, so it would not be a useful replacement assay, especially for infants taking antiretroviral prophylaxis. The Gen-Probe Aptima assay is an adequate replacement option for infant diagnosis using DBS.

Background

Diagnosis of HIV infection in infants requires detecting components of the virus since exposed infants receive anti-HIV antibodies from their mothers. For many years the gold standard for HIV-1 diagnosis in children under 18 months of age was the Roche Amplicor HIV-1 Qualitative DNA assay, version 1.5 (Roche Diagnostics, Indianapolis IN; abbreviated Roche DNA)[1], a manual assay requiring just a thermocycler and an ELISA plate reader. However, this assay is being phased out (Roche announcement dated April 17, 2014), thus a replacement infant diagnosis assay is needed, especially one for use with dried blood spots (DBS). DBS do not require phlebotomy or cold-chain storage, are easy to collect and transport to central laboratories in resource-limited settings, and have been validated for both HIV PVL monitoring and infant diagnosis [2-24].

Abbott has developed a qualitative HIV-1 assay for their m2000 platform which detects both HIV-1 RNA and DNA. We previously compared the Roche DNA to the FDA-cleared Gen-Probe Aptima HIV-1 Qualitative assay (Hologic Gen-Probe, San Diego CA) [25], and included that platform in this comparison. WHO recommends that HIV-1 virological assays used for early infant diagnosis should have sensitivity of at least 95% and specificity of at least 98% [26]. We assessed the performance of each of the assays to those targets.

Objective

Compare the Abbott qualitative assay and the Gen-Probe Aptima assay to the gold standard Roche DNA assay, using DBS generated from patients from our local study population.

Study design

DBS were prepared from EDTA whole blood using 50μl spotted on Whatman 903 cards (GE Healthcare Biosciences, Pittsburgh PA). To assess specificity, DBS were tested from 50 HIV-exposed uninfected infants from North Carolina who were screened previously for HIV infection (Roche DNA using pellets from 0.2 ml whole blood). Sensitivity was assessed using DBS from HIV-1-infected adults from North Carolina who were tested for HIV plasma viral load (PVL), since perinatal HIV transmission in North Carolina is currently rare (S. Fiscus, unpublished observation). DBS from 274 HIV-1-infected adults were tested with the Roche DNA and Aptima assays, 152 of the 274 were also tested with Abbott Qual. Depending on the HIV RNA assay used at the time of collection of the HIV-positive DBS, the lower limit of quantitation was either 400 or 40 cp/ml. Quantifiable PVL ranged from 49 to >750,000 cp/ml, plus 23 specimens with PVL under the limit of detection/quantitation.

The Abbott protocol requires 2 whole spots [27-34], but our previous validations of Roche DNA and Aptima used only two 6mm punches (approximately 35ul of blood or 17ul of plasma) since DBS samples are sometimes inadequate to allow testing of 2 entire spots. We used two 6mm punches for each assay in this comparison. DBS punches were rocked at ambient temperature and extracted according to in-house protocols for each assay. For the Roche DNA, punches were rehydrated in 1ml Roche Specimen Wash solution (BLD WS) for 1 hr; wash solution was discarded and the punches were extracted as for DNA pellets but with 15 min incubations. For the Aptima, punches were eluted in 0.525ml elution buffer [25] for 2 hr and 0.5ml of eluate was used for extraction. For the Abbott Qual, punches were eluted in 1.3ml RNA lysis buffer (Promega, Madison WI) for 2 hr; 1.2ml of eluate was loaded onto the m2000sp for total nucleic acid extraction. McNemar's test was used for statistical comparison of pairwise assay results from the same DBS.

Results

Specificity estimated among 50 HIV-negative infants was high for all 3 assays (98-100%, Table 1); the one false-positive Abbott result was negative when re-run on the Abbott assay. Sensitivity estimates varied significantly by assay (Table 1, $p<0.001$ for all assay comparisons). Five of 274 samples were excluded because valid results were not obtained from Roche DNA or Aptima (Roche: 2 indeterminate results, Aptima: 3 not tested due to insufficient specimen). Data from all three assays are plotted in Figure 1. Roche DNA was the most sensitive (96.7%), followed by Aptima (87.0%), with Abbott being the least sensitive (66.9%), with similar sensitivities when restricted to the 151 samples tested on all three assays. Sensitivity increased with PVL; for PVL <400 cp/ml (including not detected by the PVL assay), sensitivity ranged from 92.8% (Roche) to 41.7% (Abbott); sensitivity improved to 98.8% (Roche), 96.5% (Aptima), and 89.9% (Abbott) among samples with PVL >400 cp/ml. The Abbott assay only reached 95% sensitivity when PVL was above 1000 cp/ml.

Discussion

WHO recommends that HIV-1 virological assays used for early infant diagnosis have sensitivity ≥ 95% and specificity ≥ 98% [26]. All three assays achieved the targeted specificity (98-100%), but only the Roche DNA assay was sufficiently sensitive over the observed range of PVL. The Aptima assay was next best with sensitivities of 87% overall, and 96.5% among samples with PVL >400 cp/ml. The Abbott assay only reached the WHO recommended sensitivity with PVL >1000 cp/ml samples.

The lower sensitivity of the Abbott assay was surprising since it is designed to measure total nucleic acid, and the other assays were designed to measure either DNA (Roche DNA) or RNA (Aptima); Aptima may have increased signal due to cellular HIV RNA in infected blood cells, but it was still not as sensitive as the Roche DNA. The automated Abbott extraction requires "dead volume" that is not extracted by the robot, which could affect sensitivity. The Promega Lysis Buffer may not be optimal for total nucleic acid extraction, although it worked well for RNA extraction of DBS on the same platform [35]. There are several published studies using Abbott Lysis buffer for DBS elution prior to extraction for

the Abbott viral load assay [27-30,32,33], although these studies were of RNA extraction and may not be useful comparisons to Abbott total nucleic acid extraction. While we only used two 6mm punches rather than the recommended 2 whole spots, it is clear that the Abbott assay is not as sensitive as the other two assays tested here with equal sample volume. More recent proficiency testing data indicate that using a whole spot increases sensitivity of the Roche and Aptima assays (data not shown).

The Abbott assay was highly sensitive when samples with PVL >1000 cp/ml were evaluated, which was more sensitive than in the original evaluation of this assay with DBS [31]. However, infected infants may have very low viral loads (as low as 280 cp/ml in one study [36]), especially in the few weeks after birth and if they or their mothers have been treated with antiretrovirals [36-40]. Therefore, we do not recommend the Abbott Qualitative HIV-1 assay using DBS for use with infants receiving antiretrovirals as prophylaxis.

Among DBS with PVL >400cp/ml, the Gen-Probe Aptima performed well (96.5% sensitivity) in this study. Our previous results with the Aptima assay indicated sensitivity in DBS from 39 infected adults down to 20-200 cp/ml [25]. In that same study we tested DBS from 129 infected infants from several different countries and 162 exposed, uninfected infants and found 99.2% sensitivity and 100% specificity [25] despite the fact that some of the DBS had been stored under less than ideal conditions. Differences between infant and adult DBS may account for the different sensitivities of our two studies. Taking into consideration data from this work and our previous study, we have decided to replace the Roche DNA assay with the Gen-Probe Aptima assay using an entire spot.

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Highlights

- The gold standard infant diagnosis assay for HIV is being discontinued.
- An alternative assay for early infant diagnosis of HIV is needed.
- Dried blood spots (DBS) are an important specimen type for infant diagnosis of HIV.
- We compared three nucleic acid-based HIV diagnosis assays using DBS.
- One of the two new assays is a good alternate for the gold standard assay with DBS.

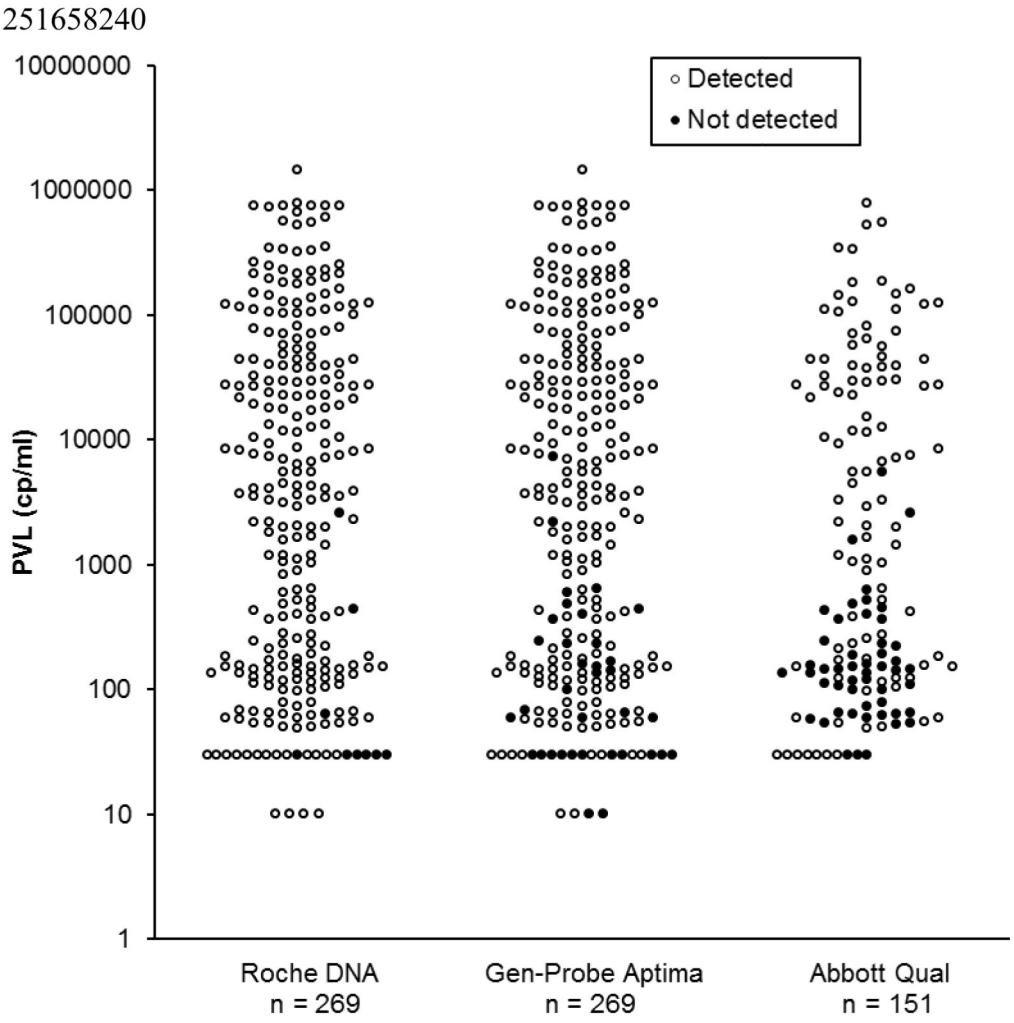

Figure 1.

Results from each assay with DBS from HIV-positive adults, plotted against PVL. Each specimen is plotted in the same relative position for the three assays. Missing dots were not tested on the Abbott assay. Results plotted at 30 cp/ml had PVL of <400 cp/mL on the Roche Amplicor HIV-1 Monitor version 1.5, standard protocol; results plotted at 10 cp/ml had PVL <40 cp/ml (detected or not) on the Abbott RealTime HIV-1 assay.

Table 1

Characteristics of three assays using DBS.

Specificity among DBS from HIV-exposed uninfected infants			
	Results (Estimate) [95% confidence interval][a]		
	Roche DNA	**Gen-Probe Aptima**	**Abbott Qual**
Specificity	50/50 (100%) [92.9-100]	50/50 (100%) [92.9-100]	49/50 (98.0%) [89.4-99.9]

Sensitivity among DBS from HIV-infected adults			
All results	260/269 (96.7%) [93.7-98.5]	234/269 (87.0%) [82.4-90.8]	101/151 (66.9%) [58.8-74.3]
Subset with results from all 3 assays[b]	148/151 (98.0%) [94.3-99.6]	128/151 (84.8%) [78.0-90.1]	101/151 (66.9%) [58.8-74.3]
Subset with PVL <400 cp/ml or not detected	90/97 (92.8%) [85.7-97.0]	68/97 (70.1%) [60.0-79.0]	30/72 (41.7%) [30.2-53.9]
Subset with PVL >400 cp/ml	170/172 (98.8%) [95.9-99.9]	166/172 (96.5%) [92.6-98.7]	71/79 (89.9%) [81.0-95.5]
Subset with PVL >1000 cp/ml	159/160 (99.4%) [96.6-100]	158/160 (98.8%) [95.6-99.8]	67/70 (95.7%) [88.0-99.1]

DBS = dried blood spot. PVL = plasma viral load.

[a] Clopper-Pearson exact 95% confidence interval.

[b] McNemar's exact test among paired sensitivity results: Roche vs. Aptima ($p<0.001$), Roche vs. Abbott ($p<0.001$), Aptima vs. Abbott ($p<0.001$)