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Detection of Low Levels of Human Immunodeficiency Virus (HIV) May Be Critical for Early Diagnosis of Pediatric HIV Infection by Use of Dried Blood Spots[∇]

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We compared a DNA-based assay with a total nucleic acid-based assay for early detection of infant human immunodeficiency virus (HIV) infection. The codetection of DNA and RNA did not result in an overall higher sensitivity compared to that of DNA alone. Discordant results were associated with low levels of HIV DNA, indicating that the sample amount may be critical.

Early diagnosis of infant human immunodeficiency virus (HIV) infection is of clinical (16) and programmatic (1) importance. However, few assays have been tested for their ability to detect HIV early after transmission from dried blood spots (DBS), which is the preferred sample collection method in developing countries (15). Since levels of RNA are generally higher than those of DNA (3), and since studies of whole blood (2, 7, 13, 14) suggested enhanced detection, we hypothesized that the detection of total nucleic acid (TNA; RNA and DNA combined) would be more sensitive for early HIV diagnosis from DBS than that of DNA alone.

To test this hypothesis, we retested DBS collected from HIV-exposed infants in Zambia (5, 6) with a recently developed TNA assay. This assay performed exceedingly well on infant samples collected in Cameroon and Uganda (11), but it had not been fully evaluated for its ability to detect HIV infection early after breast milk transmission in infants receiving single-dose nevirapine.

DBS were collected at delivery, at 1 week, and at 1, 2, 3, 4, 4.5, 5, 6, 9, 12, 15, 18, 21, and 24 months postpartum using S&S903 filter paper (Schleicher & Schuell, Keene, NH). Initial testing was conducted using a DNA-based assay for the detection of HIV from DBS as follows: a spot, consisting of 50 μl of blood, was extracted with Chelex (18) and assayed using real-time PCR (TaqMan Universal PCR master mix; Applied Biosystems, Foster City, CA) for the HIV-1 long terminal repeat gene and the beta-globin gene (17). Beta-globin gene amplification was performed in parallel on the same DNA extract to ensure an adequate number of cells in the DBS. Four- and 24-month samples were batch tested, and if they were positive, the timing of transmission was determined by systematically testing earlier samples (6). Positive results were confirmed by a second sample from a later time point. At delivery, single-dose nevirapine was provided to participants.

To test if the TNA assay might allow an earlier detection of HIV infection, we selected the last DNA-negative (DNA^-) and first DNA-positive (DNA^+) sample (median difference, 28 days; range, 7 to 140) from 94 infants. We also included 46 samples of infants who tested positive at delivery, as well as 50 4-month samples of infants who were negative throughout the 24-month follow-up. DBS were reextracted and tested with the TNA assay at the CDC (Atlanta) as previously described using about one-fifth of the DBS (corresponding to about 10 μl of blood) (11). DBS had been stored with desiccant at room temperature for a median of 42 months (range, 16 to 61 months) when tested using the TNA assay and a median of 29 months (range, 9 to 47 months) after the DNA testing.

Results of the TNA assay were available for 280 (99%) samples; four results (three DNA^+ and one DNA^-) were missing due to an insufficient amount of sample. Among the samples with available results, 5 (5%) of 93 of the last DNA^- samples collected from birth to 18 months were TNA positive (TNA^+) and 9 (10%) of 92 of the first DNA^+ samples collected from 1 week to 21 months were TNA negative (TNA^-). When restricted to one (i.e., the earliest positive) sample per infant, the sensitivity of the DNA-based assay was slightly, but not significantly, higher than that of the TNA assay (95% versus 90%; McNemar test; $P = 0.19$). In addition, the TNA

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TABLE 1. Detection of HIV-1 by DNA-based and TNA-based real-time PCR analyses from DBS collected from Zambian infants[a]

Infant age	Last DNA^- sample		First DNA^+ sample	
	No. of TNA^-/DNA^- (%)	No. of TNA^+/DNA^- (%)	No. of TNA^-/DNA^+ (%)	No. of TNA^+/DNA^+ (%)
Delivery	5/5 (100)	0/5 (0)	7/45 (16)	38/45 (84)
1 wk	42/43 (98)	1/43 (2)	1/4 (25)	3/4 (75)
1 mo	14/14 (100)	0/14 (0)	1/44 (2)	43/44 (98)
2 mo	3/4 (75)	1/4 (25)	1/15 (7)	14/15 (93)
3–6 mo	16/19 (84)	3/19 (16)	2/14 (14)	12/14 (86)
9 mo or later	8/8 (100)	0/8 (0)	4/15 (27)	11/15 (73)
Total	88/93 (95)	5/93 (5)[b]	16/137 (12)	121/137 (88)[c]

[a] The table excludes four samples (one DNA^- and three DNA^+) without available TNA results.
[b] Exact binomial (95% CI, 2 to 12%).
[c] Asymptotic binomial (95% CI, 83 to 94%).

assay failed to detect 7 (16%) of the HIV-positive birth samples and was positive for 1 (2%) of the 50 negative 4-month samples, resulting in an overall sensitivity of 88% (95% confidence interval [CI], 83% to 94%) and a specificity of 98% (95% CI, 89% to 100%) compared to the DNA assay. Overall, both assays were discordant in the timing of transmission for 21/137 (15%) infants (Table 1).

Failure of the TNA assay to detect a positive DNA result was associated with low levels of proviral DNA as measured in the DNA real-time PCR. Median proviral DNA levels were 502 (interquartile range [IQR], 162 to 1,473) and 2,091 (IQR, 802 to 6,373) copies per 1,000,000 cell equivalents (Wilcoxon rank-sum test; $P = 0.006$), respectively, for TNA^-/DNA^+ ($n = 16$) and TNA^+/DNA^+ ($n = 117$) specimens. Data were missing from 4 (3%) of the 137 DNA^+ specimens. TNA assay failure was not associated with the duration between the performance of the two assays, given the median duration of 31 (IQR, 19 to 42) and 28 (IQR, 16 to 40) months (Wilcoxon rank-sum test; $P = 0.73$), respectively, for TNA^-/DNA^+ ($n = 16$) and TNA^+/DNA^+ ($n = 121$) specimens.

Contrary to our expectations, the TNA assay did not detect HIV infection earlier than the DNA assay. This may be explained by the age of the sample and the possible degradation of RNA (9), which is less stable than proviral DNA (8, 10). It should be noted that the TNA assay was able to detect five samples at an earlier time point, possibly indicating variations in the RNA/DNA ratio per sample and a slightly higher sensitivity of the TNA assays in at least some cases.

More importantly, our data suggest that the detection of low levels of HIV may be critical for the early HIV diagnosis. No statistical association of the TNA-negative results with the time between assays was found; however, we cannot exclude that sample age contributed to the difference between the assays. These results should therefore be repeated using fresh samples. We also cannot exclude that mutations in the primer and probe binding regions or that false positivity in the DNA assay contributed to differences between the two assays. However, given the good performance of the DNA assay in our hands and the TNA assay in an earlier evaluation (11), the latter arguments are unlikely to explain the large differences observed here.

While the TNA assay used only about one-fifth of the input of the DNA assay, this difference was at least partly compensated by a higher extraction volume in the DNA assay (80 to 100 μl versus 55 μl in the TNA assay) and a lower input in the PCR (5 μl versus 10 μl). The DNA assay's ability to detect low levels of HIV may be due to differences in extraction efficiencies.

In summary, we found a large number of discordant samples between both assays. This is in concordance with previous publications that generally demonstrated a low sensitivity shortly after HIV exposures (4, 12). Given the clinical relevance of early infant HIV diagnosis, the ability of assays to detect low levels of HIV may be important.

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