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Ultrasensitive p24 Antigen Assay for Diagnosis of Perinatal Human Immunodeficiency Virus Type 1 Infection▽

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We evaluated an ultrasensitive p24 antigen enzyme immunosorbent assay on 802 plasma specimens from 582 infants and children of 0 to 180 days of age. Overall sensitivity and specificity were 91.7% and 98.5%, respectively. After exclusion of infants of less than 7 days of age, the sensitivity and specificity were 93.7% and 98.3%, respectively.

Antibody assays cannot be used to diagnose perinatal human immunodeficiency virus (HIV) infection until after maternal antibodies have waned at 12 to 18 months. In industrialized countries, infants are tested repeatedly during the first 12 to 18 months of life with nucleic acid amplification tests such as HIV DNA or RNA PCRs (2, 5, 15, 20, 28, 32). However, PCR assays are technologically complex, requiring expensive kits and equipment as well as highly trained technologists. Limited budgets and infrastructure in resource-constrained settings require the cost-effective use of laboratory tests. Given these concerns, the WHO strongly encourages the development of technologically simpler, less expensive assays that can be used to diagnose HIV infection in early infancy (31).

One simpler method that is gaining support for the detection of HIV infection in infants is the heat-denatured, signal-amplified p24 (ultrasensitive p24 [Up24]) antigen assay (26). The assay requires only an enzyme immunosorbent assay washer, a reader, a heat block, and an incubator, as opposed to the thermocycler required by nucleic acid technologies. We evaluated the sensitivity and specificity of this assay for early-infancy diagnosis of HIV infection using stored plasma specimens from HIV-exposed infants of 0 to 180 days of age from two U.S. cohorts: the North Carolina Children's AIDS Network cohort (9–11) (n = 154) and the New York City Pediatric AIDS Collaborative Transmission Study (PACTS) cohort (1, 4, 13, 28, 29) (n = 648). Selection criteria included sufficient volume to perform the testing. The children's HIV status had been previously determined by a combination of criteria, including Roche Amplicor HIV DNA testing (version 1.0) and/or HIV culture, as previously described (4, 9–11, 13, 20, 28). Nine of 125 (7%) children in the North Carolina cohort were known to be infected, along with 100 of 457 (22%) children in the PACTS cohort.

(Institutional review boards of Columbia University, the Medical and Health Research Association of New York City, and the Centers for Disease Control and Prevention approved the original PACTS study, which included blood storage for future studies. Specimens from North Carolina were obtained for clinical diagnostic purposes. This work was considered exempt by the institutional review boards of the University of North Carolina and the Centers for Disease Control and Prevention, as excess samples were stripped of identifying information and delinked prior to receipt at the University of North Carolina.)

TABLE 1. Total numbers of positive HIV DNA or culture assay and Up24 antigen assay results compared to final infection status

Subject age (days)	HIV DNA result				Up24 antigen result			
	No. of positive samples/total no. tested		Sensitivity	Specificity	No. of positive samples/total no. tested		Sensitivity	Specificity
	HIV$^+$	HIV$^-$			HIV$^+$	HIV$^-$		
0–7	2/5	0/109	0.387	0.9995	0/5	1/109	—[a]	0.999
8–30	21/25	1/141	0.857	0.995	25/29	3/151	0.900	0.987
31–90	74/83	2/226	0.897	0.993	88/94	5/273	0.933	0.980
91–180	32/33	0/69	0.943	0.988	44/45	1/96	0.941	0.977

[a] —, could not be determined accurately due to the small numbers of samples tested.

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Plasma specimens were tested for HIV-1 p24 antigen with the Perkin Elmer (Boston, MA) Life Science NEK 050B HIV-1 p24 ELISA kit, the Perkin Elmer NEP116VL ELAST amplification system, and Quanti-Kin Detection System software (Diagnostica Ligure s.r.l, Genoa, Italy), according to the Up24 assay protocol provided by the manufacturer. Sensitivity, specificity, and positive and negative predictive values for the Up24 and HIV DNA assays were determined (25). The final infection status of the child was used as the "gold standard." Given that multiple specimens per child were tested, these results were computed from repeated measures models fit with generalized estimating equations (GEE) (33). Sensitivity and specificity were computed from a GEE model with the assay result as the dependent variable; positive and negative predictive values were computed from a GEE model with final infection status as the dependent variable. An exchangeable correlation structure was used for all models. All data analyses were performed with SAS software, version 9.1 (SAS Institute Inc., Cary, NC).

We tested 802 specimens from 582 children ranging in age from 0 to 180 days of life (Table 1) in the Up24 antigen assay and 691 specimens from 524 infants in the DNA assay. Overall sensitivity and specificity were 91.7% and 98.5%, respectively, for the Up24 antigen assay, compared to 88.3% and 99.4% for the DNA assay (Table 2). Excluding infants of less than 1 week of age improved the sensitivity (93.7%) and positive and negative predictive values and decreased the specificity slightly (98.3%) for the Up24 antigen assay and improved the sensitivity of the DNA assay. The sensitivity and negative predictive value were slightly better for the Up24 antigen assay, while the specificity and positive predictive value were somewhat better for the DNA assay, though these differences were insignificant.

There were 16 false-negative results from 11 individual children among 173 samples from infected infants tested with the Up24 antigen assay. Two children had two specimens each and one child had four different specimens from different time points that were negative in the assay. The median age for specimens with false-negative results was 24 days (range, 0 to 145 days). Specimens with false-positive results in the Up24 antigen assay came from children with a median age of 37 days (range, 3 to 111 days), although most (8 of 10) were in the 19- to 41-day range. In the DNA assay, there were 17 false negatives from 16 individual children among 146 samples tested from children who were infected. The median age for specimens with false-negative results in the DNA assay was 35 days (range, 1 to 104 days). Only three false positives were observed in the DNA assay.

Lyamuya et al. (16) demonstrated that even an early research version of the heat-denatured p24 antigen assay was 99% sensitive and 100% specific in diagnosing HIV subtype A and D infections in 231 samples from 177 children in Tanzania. Similar results have been observed with subtype B in Switzerland (87% sensitivity and 99% specificity [n = 873]) (19), subtype E in Thailand (100% sensitivity and 100% specificity in 142 samples) (30), subtype C in South Africa (n, 203 [27] and 141 [21]) and Zimbabwe (n = 164 [34] [97 to 98% sensitivity and 97 to 99% specificity]), and multiple subtypes from the Democratic Republic of Congo with either plasma (n = 150) (sensitivity, 92.3%; specificity, 100%) or dried plasma spots (n = 87) (sensitivity and specificity, 100%) (6). Our data con-

TABLE 2. Overall sensitivity and specificity results for HIV DNA assay and Up24 antigen assay for infants 0 to 180 days old and 8 to 180 days old compared with final infection status

Subject age (days)	Result (95% confidence interval)[a]							
	Up24 with kit buffer				HIV DNA assay			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
0–180	0.917 (0.852–0.955)	0.985 (0.971–0.992)	0.940 (0.891–0.968)	0.975 (0.951–0.987)	0.883 (0.821–0.926)	0.994 (0.984–0.998)	0.977 (0.931–0.993)	0.970 (0.950–0.982)
8–180	0.937 (0.876–0.970)	0.983 (0.968–0.991)	0.946 (0.898–0.972)	0.979 (0.955–0.990)	0.900 (0.842–0.938)	0.993 (0.979–0.998)	0.977 (0.930–0.993)	0.969 (0.948–0.981)

[a] Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were all estimated from a repeated-measures model with GEE. The numbers of samples tested are as follows: Up24 with subjects of 0 to 180 days old, 802; Up24 with subjects of 8 to 180 days old, 688; HIV DNA assay with subjects of 0 to 180 days old, 691; HIV DNA assay with subjects of 8 to 180 days old, 577.

firm these previous studies in a very large group (802 samples) of HIV-exposed infants in the United States.

There has been some concern about the specificity of the Up24 assay with very young (less than 1 month old) infants, as the p24 antigen might cross the placenta and be detected (24). However, this may primarily be a problem with the acid-dissociated immune complex-disrupted assay and was not observed by other investigators (3, 17, 18, 22, 23). Many of the previous studies assessing the Up24 antigen assay sampled only children who were at least 6 weeks old (27) or did not discuss in detail the number of samples from those less than 1 month old (6, 30, 34). We tested 109 specimens from infants of 0 to 7 days of age and 151 specimens from infants of 8 to 30 days of age and had only four false positives. Sensitivity increased with age (Table 1), similar to the increased sensitivity observed in both HIV nucleic acid assays and HIV cultures (3, 7, 8). Given limited resources, the most useful time for using virologic assays to diagnose pediatric HIV infection is between 6 and 14 weeks of age, which corresponds to regularly scheduled immunization visits and allows early diagnosis for entry into treatment.

Many field investigators cite the ease of collecting and transporting dried blood spots (DBS) (12), as opposed to phlebotomy of very young children and transportation of whole blood or plasma. Although nucleic acid extraction, amplification, and detection become technologically more complex and expensive with DBS than with whole blood or plasma specimens, widespread access for infant testing will undoubtedly require their use. Patton et al. (21) and Knuchel (14) have recently demonstrated that DBS can be used for Up24 antigen detection. Optimization of this assay for DBS in order to make infant diagnosis of HIV infection feasible in district and provincial hospitals and clinics in all resource-limited countries should be a high priority.

The limitations of this study include the fact that only children with subtype B infection were tested, although, as mentioned above, the assay has performed well with a variety of subtypes (16, 27, 30, 34), with the possible exception of subtype D (14). In addition, all tests were performed in the United States by a technologist with 10 years of experience with different versions of the assay. However, others have successfully transferred this technology to several resource-limited settings (16, 28, 31, 34). The strengths of the study include its very large sample size (802 specimens from 582 children), especially of infants less than 30 days of age.

We found the commercially available Up24 antigen assay to be sensitive and specific for diagnosing perinatal HIV infection in infants. These results and those of others (6, 16, 19, 21, 27, 30, 34) support the use of the assay in regions where DNA PCR is not readily available.

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