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Sensitivity of Immune Complex-Dissociated p24 Antigen Testing for Early Detection of Human Immunodeficiency Virus in Infants

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Several investigators have suggested that early diagnosis of human immunodeficiency virus (HIV) infection in infants could be accomplished with a modified, more-sensitive, acid-dissociated p24 antigen enzyme-linked immunosorbent assay (ELISA) technique (p24 antigen immune complex dissociation [ICD]). We compared detection of HIV infection by HIV culture, PCR, and p24 antigen ICD assays in 46 infants by using samples collected independently. The detection sensitivity of the p24 antigen ICD assay was 0% with cord blood samples (2 HIV-positive infants), 38% with plasma samples from infants under 3 months of age (8 HIV-positive infants), and 58% overall (12 HIV-positive infants). By contrast, the sensitivities of HIV culture and PCR were 50% for cord blood samples, 75% for plasma samples from infants under 3 months of age, and 83% overall. These results indicate that the p24 antigen ICD does not offer the sensitivity necessary for this assay to be used as an indicator of HIV infection in infants.

Early diagnosis of human immunodeficiency virus (HIV) infection in infants born to HIV-infected mothers is complicated by the presence of maternal anti-HIV immunoglobulin G antibody which renders standard serologic testing by enzyme-linked immunosorbent assay (ELISA) and Western blot (immunoblot) of little diagnostic significance. Several groups, including our own, have found that HIV culture and PCR offer excellent sensitivity and specificity for diagnosis of HIV infection in early infancy (3, 4, 6, 7, 13). Unfortunately, these tests in the first 3 months of life are not able to detect all of the babies who eventually prove to be HIV infected (8, 19, 21). This may be due to the timing of HIV transmission or to the size of HIV inoculum that the infant initially received.

Recently, several investigators have advocated the diagnostic use in infants of a p24 antigen detection method based on immune complex dissociation (ICD) by acid treatment of plasma or serum (2, 8–11, 15, 16, 18, 19, 21, 23). Because several centers propose to use the p24 antigen ICD assay to provide such information, it is important to evaluate it in comparison with other methods already proven by several investigators to be useful. This study compares HIV culture, PCR, and p24 antigen ICD using independent samples in cases where the HIV infection status is known.

MATERIALS AND METHODS

Study population. The study population consisted of 75 infants born to HIV-infected mothers who were evaluated at the Pediatric-Obstetrical HIV Research Center at Baylor College of Medicine between January 1990 and December 1993. This included 10 infants reported in a study by Kline et al. (13). Four specimens were cord blood, four were from the less-than-3-month-old group of infants, and two were from the total sample group. Written informed consent was obtained from the mother of each infant for collection of cord blood and subsequent infant blood samples. The Centers for Disease Control pediatric classification system was used for determination of infant infection status (5). HIV-positive infants had positive ELISA and Western blots after 15 months of age. HIV cultures, HIV PCR, and p24 antigen ICD ELISA assays were performed in separate laboratories by investigators who were blinded to the infection status of the infant. The infection outcome of the infant had to be known to be included in the comparison of laboratory predictors.

HIV cultures. All HIV cultures were performed by a National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group consensus protocol (12), with modification for smaller blood volumes. Briefly, cultures were performed in duplicate in a 24-well tissue culture plate with six fivefold serial dilutions beginning with 10^6 patient peripheral blood mononuclear cells. Each sample of patient cells was cocultured with phytohemagglutinin-stimulated normal donor peripheral blood mononuclear cells for 14 days. The supernatant from each individual well was assayed for viral expression of HIV p24 antigen by a standard ELISA. A microculture well was scored positive if p24 antigen was present in a concentration of 30 pg/ml or greater.

PCR. PCR was performed by previously described procedures (13, 17, 20, 22). Briefly, an HLA-DQ gene primer set and two HIV *gag* gene primers were used to analyze the DNA. The HLA-DQ PCR was performed to ensure that the DNA in the sample was sufficient and amplifiable.

The procedure for PCR with *gag* primers 225 to 775 (22) included an initial PCR cycle at 94°C for 3.5 min, 55°C for 1 min, and 72°C for 2 min and cycles 2 through 40 at 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min. The products (500 bp) were separated on agarose gels and transferred to zeta-probe membranes for probing with a ^{32}P-end-labeled 22-bp oligonucleotide (positions 700 to 721). The PCR procedure with *gag* primers SK38 and SK39 differed in that cycling was at 95°C for 4 min, 94°C for 1 min, 55°C for 2 min, 72°C for 2 min, and 72°C for 7 min for 40 cycles. The 115-bp product was detected with SK19 (positions 1595 to 1635) (17).

Infants were considered to be PCR test positive if an amplified fragment of expected length was detected by specific hybridization in duplicate tubes in at least two separate experiments with one or more primer sets. Because the false-positive results were confirmed by multiple testing of the sample, the sample was assumed to be contaminated. Subsequent PCR testing of later samples from the infants were negative.

Acid-dissociated p24 antigen ELISA. Plasma was stored at −70°C until use. The plasma was thawed and then assayed for p24 antigen with the Coulter ICD-Prep kit as described in the manufacturer's instructions (18). Briefly, 100 μl of each plasma tested was added to wells, and then 100 μl of 0.15 M glycine HCl plus Triton X solution was added. The wells were incubated for 90 min at 37°C, Tris reagent was added to neutralize the reaction, and the p24 ELISA was performed by transferring 200 μl to a p24 antigen plate. The plates then were sealed and incubated overnight at 37°C. Negative controls were used to calculate a mean absorbance. The cutoff value for positivity was the negative mean absorbance plus 0.055. The cutoff value for the assays performed was never above 0.12, as was described in the original publication (15). The results were quantitative and ranged between 35 and 250 pg/nl. No hemolyzed specimens

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TABLE 1. Comparison of p24 antigen ICD, HIV culture, and PCR in early detection of HIV infection

Test	Detection of HIV infection in samples[a]											
	Cord blood (n = 19)				Plasma from <3-mo-old infants (n = 30)				Total sample (n = 46)			
	Sensitivity		Specificity		Sensitivity		Specificity		Sensitivity		Specificity	
	Positive infants detected[b]	% (CI)[d]	Negative infants detected[c]	% (CI)	Positive infants detected	% (CI)	Negative infants detected	% (CI)	Positive infants detected	% (CI)	Negative infants detected	% (CI)
HIV Culture	1/2	50 (3–97)	17/17	100 (77–100)	6/8	75 (36–96)	22/22	100 (82–100)	10/12	83 (51–97)	34/34	100 (87–100)
PCR	1/2	50 (3–97)	16/17	94 (69–100)	6/8	75 (36–96)	21/22	95 (75–100)	10/12	83 (51–97)	32/34	94 (79–99)
p24 Antigen ICD	0/2	0	15/17	88 (62–98)	3/8	38 (10–74)	20/22	91 (69–98)	7/12	58 (29–84)	29/34	84 (66–94)

[a] Nineteen cord blood specimens were tested, 11 more specimens from infants under 3 months of age were tested, and 16 specimens from infants greater than 3 months but less than 34 months were tested.
[b] Number of positive infants detected/total number of positive infants.
[c] Number of negative infants detected/total number of negative infants.
[d] Ninety-five percent confidence intervals (CI) are shown in parentheses.

were analyzed. Specimens found to be falsely positive were reexamined in multiple assays.

Statistical analysis. The operational characteristics of a diagnostic test, sensitivity and specificity, were calculated, and confidence intervals for these point estimates were estimated by the binomial distribution (1). These intervals provide the range of values likely to cover the true but unknown value of the characteristic. The McNemar test was used to determine concordance between the regular p24 and p24 antigen ICD assays and the duplicate assays (14).

RESULTS

Same-specimen comparison of p24 and p24 antigen ICD assays. To verify results of several investigators, we compared 30 plasma samples by both p24 and p24 antigen ICD assays. Fourteen samples came from infants who were found subsequently to be HIV uninfected, eight samples were from HIV-infected infants, and eight specimens were from infants whose current infection status is classified as indeterminate. In the samples from HIV-infected infants, six of eight babies were positive by the regular p24 antigen assay; however, seven of eight babies were positive by the p24 antigen ICD assay. The outcomes for the two assays did not differ statistically ($P \geq 0.25$). Compared with the regular p24 antigen assay, there was a 2- to 250-fold increase in p24 antigen detection by the ICD method depending on the specimen (mean fold increase in p24 antigen, 51 ± 98). In one case, both p24 antigen detection methods were off scale when undiluted plasma was used; in another case, both regular and p24 antigen ICD assays were negative. Of the samples from the babies who were found subsequently to be uninfected, no p24 antigen was detected by either assay in 12 of 14 tested. However, in two cases, the p24 antigen ICD test was falsely positive. Analysis of the eight plasma samples tested from the indeterminate infants showed that there was no p24 antigen detected by either assay.

Repeat specimens for p24 antigen ICD. Plasma specimens from 12 infants were split and tested in duplicate or triplicate with separate assay times. Ten of these specimens were from noninfected infants; two were from HIV-infected infants. Concordance between specimens occurred in 10 of 12 cases. The results did not differ statistically ($P \geq 0.48$). In the two disparate cases, one sample was taken from an HIV-infected infant who initially tested positive and then tested negative in two repeat tests; the other sample was from an HIV-uninfected infant who was negative in the first assay, positive in the second, and negative in a third.

p24 Antigen ICD detection. The p24 antigen ICD test was performed on 105 specimens from 75 infants. Of the 75 infants, samples from 46 infants met the inclusion criteria of this study. These criteria were: (i) samples were independent, (ii) the infection status of the infant was known, and (iii) results were available for all three tests, i.e., HIV culture, PCR, and p24 antigen ICD. Twelve of the 46 infants in the sample proved to be HIV infected, and 34 were uninfected, giving an infection rate of 26%, which is consistent with our previously published data with a larger data base (13). The range of ages tested was 0 to 34 months; 37 of 46 infants were under 6 months of age, and 30 of 46 infants were 3 months or younger. Nineteen of the 46 specimens were from cord blood. In this group, as shown in Table 1, the p24 antigen ICD assay did not identify either one of the two HIV-infected infants, for a sensitivity of 0%. In addition, there were two false-positive test results, reducing the specificity to 88%. A single false-positive test result was seen with PCR (specificity, 94%), and no false positives occurred with HIV culture (specificity, 100%). PCR and HIV culture were positive in one of two infants who subsequently became HIV infected, giving a sensitivity for each assay of 50%.

Examination of the plasma from infants under 3 months of age included 30 specimens, including the 19 cord blood specimens. The results shown in Table 1 indicate that the sensitivity of the p24 antigen ICD assay was 38%, well below the 75% found for both culture and PCR. HIV culture and PCR identified six of eight HIV-infected infants, whereas the p24 antigen ICD assay identified only three of eight. The specificity of the HIV culture was 100%, whereas one false-positive and two false-positive test results occurred with the HIV PCR and p24 antigen ICD assay, respectively. This gave the PCR a 95% specificity and the p24 antigen ICD test a 91% specificity.

Plasma samples from an additional 16 infants more than 3 months of age at the time of analysis were tested. When all of the samples from 46 infants are considered (Table 1), the sensitivity of the p24 antigen ICD assay was 58%. However, the sensitivities of both PCR and HIV culture were 83%. The HIV culture and PCR detected 10 of 12 samples, whereas the p24 antigen ICD assay detected 7 of 12 samples. The specificities were 85% for p24, 94% for PCR, and 100% for HIV culture.

Twenty-eight additional plasma samples from infants born to HIV-infected women were analyzed for p24 by the acid-dissociated test. In four of these cases, culture was not performed. In another four, PCR was not performed, two were lost to follow-up, and 18 had indeterminate infection status. In the cases where either HIV culture or PCR was performed and

the outcome was known, there was concordance with the p24 antigen ICD results in six of eight cases. In one case, the p24 antigen ICD assay was positive in an HIV-infected infant; however, the HIV culture was negative. In the other case, the infant was not HIV infected; however, the p24 antigen ICD assay was positive. In the cases where infection status was indeterminant, 16 of 18 specimens were tested by all three assays with negative concordant results. In one case, no PCR was performed, and in another case, the PCR was positive while the HIV culture and p24 antigen ICD were negative.

DISCUSSION

These data, derived by using independent samples and comparison of proven HIV detection methods, show that the diagnosis of HIV infection by p24 antigen ICD is not as sensitive as that by HIV culture or PCR. The lack of sensitivity of the p24 antigen ICD assay was not confined to samples from infants under 3 months of age. Although the sensitivity of the p24 antigen ICD rose from 0% in the cord blood specimens to 58% overall, our results indicate that whereas the assay may be useful as an adjunct to confirm culture and PCR testing, it cannot replace these more-sensitive assays. The strength of our data is in the concurrent comparison of p24 antigen ICD with HIV culture and PCR.

In the original work of Miles et al. (15), five of eight cord blood specimens from infants with HIV infection were positive by the p24 antigen ICD test. In addition, 20 of 22 infants without HIV infection were negative by p24 antigen testing; in 2 of 22 cases, the cord blood specimen was p24 antigen positive, but subsequent specimens were negative. The authors also presented an analysis of the total sample studied, which indicated a sensitivity of 81% and specificity of 100% for 78 children from 6 days to 13 years of age. In a confirmation of the work by Miles et al., Fiscus et al. showed that five of five HIV-infected infants tested in the first 3 months of life were p24 antigen positive (10, 15). Our study tested only two cord blood samples from HIV-infected infants; however, as indicated previously, no comparison was made with other HIV detection methods in the study by Miles et al. (15). In addition, HIV culture and PCR detected six of eight HIV-infected infants; however, p24 antigen ICD detected only three of eight HIV-infected infants when they were assayed under 3 months of age. In addition, Fiscus et al. indicated that there were no false-positive test results in the 10 infants who subsequently seroreverted (10). This is in contrast to our results showing that 2 of 21 specimens were false positive with the p24 antigen ICD assay. In another study, the results of the p24 antigen ICD testing were more unpredictable and suggested that test sensitivity was similar for the p24 antigen ICD method and the standard p24 antigen assay, especially in the first few months of life (9). Our work with a same-specimen comparison of 30 samples from infants under 3 months of age confirms this early brief report. Another study, by Walter et al., compared HIV culture and p24 antigen ICD in 13 HIV-infected infants (15 samples) under 3 months of age and found concordant positive results in five cases; however, in six cases, the HIV culture was positive but the p24 antigen ICD was negative (23). In two cases, HIV culture and p24 antigen ICD results were negative initially but became positive after 3 or 4 months (23). Our results suggest a difference in detection sensitivity of twofold in the infants under 3 months of age; HIV culture and PCR are more sensitive than the p24 antigen ICD assay.

In our studies where we have emphasized early detection of infection, the p24 antigen ICD test gave variable results for the same specimens tested at different times, although the results were not statistically significant. In addition, there were more false-positive test results with the p24 antigen ICD test than observed with PCR. Results that compared testing of 30 specimens show that the amount of p24 antigen detected is enhanced by acid dissociation; however, of the eight HIV-infected specimens examined, only one additional specimen was classified as positive. This increase in detection sensitivity was not statistically significant. The conclusion that this assay is useful to classify newborns or very young infants as being infected may have been premature.

Although our study was limited to a small number of specimens from HIV-infected infants (i.e., eight) under 3 months of age, our results suggest that caution should be exercised in the use of p24 antigen ICD to diagnose early HIV infection in infants.

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