

Evaluation of a Ultrasensitive p24 Antigen Assay as an Alternative for Nucleic Acid Testing in the Diagnosis of Pediatric HIV-1 Infection in Infants

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Abstract

Background: The detection of HIV-1 by PCR is useful for the diagnosis of perinatal infection in infants. Commercially available PCR methods are based on elaborate amplification approaches, are expensive, and require skilled technicians with specialized equipment and facilities, making their use prohibitive for many resource-limited areas. An inexpensive and less complex approach for the diagnosis of HIV-1 infection in infants would be valuable in resource-limited settings. An inexpensive and more sensitive (as compared to standard p24) “boosted” p24 assay, combining heat denaturation and ELISA technology, has been previously described; however transfer of this approach has been limited due to its research nature.

Methods: A laboratory protocol, test kit and software for an ultrasensitive p24 antigen assay (Up24) were developed based on the research version. To estimate the sensitivity and specificity of Up24 for use in infant HIV-1 diagnosis, 217 specimens from HIV infected infants between 10 and 24 weeks of age from New York City and North Carolina, and 540 specimens from uninfected, HIV-exposed infants collected over a broad range of time points (including 6 and 24 weeks) of age were tested by Up24. A subset of samples was also tested with a new extraction buffer reported to improve sensitivity. Results were compared to known infection status as determined by DNA PCR.

Results: Up24 with standard kit buffer was 94.0 % sensitive (95% confidence interval (CI) = 90.0-96.8 %), 98.7 % specific (CI = 97.4-99.5 %). Assuming an HIV prevalence of 28.7%, the Up24 assay yields a positive predictive value of 96.7% (CI = 93.3-98.7 %) and negative predictive value of 97.6% (CI = 96.0-98.7 %). Use of the new extraction buffer increased Up24 sensitivity to 96.7 % (CI = 88.7-99.6 %) while specificity remained the same.

Conclusions: Up24 may be useful as an inexpensive method to diagnose HIV-1 infection in infants applicable to resource-limited settings. Research is needed to determine at what age p24 antigen is reliably detected by Up24, and to evaluate the use of Up24 on infant specimens from settings where non-B HIV-1 subtypes predominate.

Background

- Detection of HIV-1 by nucleic acid-based (NA) methods targeting DNA or RNA have proven to be useful for the early diagnosis of perinatal infection in infants.
- However, since nucleic acid methods are based on elaborate amplification approaches, they are usually expensive, requiring specialized equipment and skilled technicians.
- A less expensive and complex approach would be valuable in resource-limited settings where NA testing is prohibitive.

HIV-1 P24 Antigen

- p24 antigen rises in parallel with viral load but requires effective dissociation of antigen-antibody complex to improve sensitive detection.
- Current protocols involve using base or acid denaturation but still suffer from specificity issues.

HIV-1 P24 Antigen

- A relatively inexpensive, and more specific and sensitive, “boosted” p24 assay combining heat denaturation and signal amplification has been described previously (Schupbach et. al. *AIDS 10* 1996: 1085).
- However, it has met with limited success outside the laboratory where it was originally developed.

HIV-1 P24 Antigen

- To address this, a standardized version of the assay has been developed by Perkin Elmer Life Science termed Ultrasensitive p24 (Up24).
- Purpose of this study was to continue ongoing evaluations (Respass et. al. J. Clin. Micro. 43 2005: 506) of this assay to further support it's use in pediatric diagnosis.
 - *Use of trade names is for identification only and does not constitute endorsement by the Public Health Service, U.S. Department of Health and Human Services.*

Ultrasensitive p24 Components

- HIV-1 p24 enzyme-linked immunosorbent immunoassay kit (cat. #: NEK050; Perkin Elmer Life Sciences, Boston, MA).
- P24-specific viral load ELAST amplification system assay kit which includes Up24 assay protocol (NEP116VL; Perkin Elmer Life Sciences, Boston, MA).
- Quanti-Kin detection system software (Rilab, Genoa, Italy).

Nucleic Acid vs. Ultrasensitive p24 Assay

Characteristic	Nucleic Acid	Ultrasensitive p24
Amplification	Nucleic acid sequence/signal	Signal
Equipment needs	May require dedicated equipment	General ELISA equipment and a dry heat block
Contamination risk	High to moderate	Moderate to low
Work space needed	Moderate to extensive	Moderate
Technician Skills	Sophisticated	Moderate
Cost (Single test)	Up to 70 USD in developed countries; tiered for resource poor settings	<u><10</u> USD

Archived Specimens Tested with Up24

- A total of 749 (209 infected and 540 uninfected) archived infant samples from New York and North Carolina cohorts were tested using standard buffer extraction protocol as described in the product insert.
- A subset of 482 were tested with standard extraction protocol supplemented with a new virus lysis buffer reported to improve sensitivity of the assay.

Archived Specimens Tested with Up24

- Infection status was determined by a combination of criteria including:
 - Nucleic acid testing
 - Serology
 - Viral Culture

Laboratory Methods

- Ultrasensitive p24 determinations were done in singlet as described in the provided assay protocol .
- The testing laboratory was blinded to HIV status of infants.
- Statistical analyses were done using SAS (SAS Inc., Cary, N.C). CI is not reported within age groups when number of cases are less than 2.

Table 1: Up24 results with standard extraction buffer

<i>Time from birth (I: Infected; NI: not infected)</i>	<i>Sensitivity (95% CI)</i>	<i>Specificity (95% CI)</i>	<i>Positive Predictive Value (95% CI)</i>	<i>Negative Predictive Value (95% CI)</i>
0 – 7 days (I: 2; NI: 112)	0.000	0.991 (0.951 – 1.000)	0.000	0.982 (0.938 – 0.998)
8 – 30 days (I: 6; NI: 63)	1.000 (0.541 – 1.000)	1.000 (0.943 – 1.000)	1.000 (0.541 – 1.000)	1.000 (0.943 – 1.000)
31 – 90 days (I: 84; NI: 255)	0.952 (0.883 – 0.987)	0.980 (0.955 – 0.994)	0.941 (0.868 – 0.981)	0.984 (0.961 – 0.996)
91 – 180 days (I: 44; NI: 97)	1.000 (0.920 – 1.000)	0.990 (0.944 – 1.000)	0.978 (0.882 – 0.999)	1.000 (0.962 – 1.000)
>180 days (I: 73; NI: 13)	0.973 (0.905 – 0.997)	1.000 (0.753 – 1.000)	1.000 (0.949 – 1.000)	0.867 (0.595 – 0.983)
Overall (I:209; NI: 540)	0.958 (0.921 – 0.981)	0.987 (0.974 – 0.995)	0.967 (0.933 – 0.987)	0.983 (0.969 – 0.992)

Table 2: Up24 results with new lysis buffer protocol

<i>Time from birth (I: infected; NI: not infected):</i>	<i>Sensitivity (95% CI)</i>	<i>Specificity (95% CI)</i>	<i>Positive Predictive Value (95% CI)</i>	<i>Negative Predictive Value (95% CI)</i>
0 – 7 days (I: 1; NI: 104)	1.000	0.990 (0.947 – 1.000)	0.500	1.000 (0.964 – 1.000)
8 – 30 days (I: 0; NI: 51)	-	0.980 (0.896 – 1.000)	0.000 (0.000 – 0.975)	1.000 (0.929 – 1.000)
31 – 90 days (I: 55; NI: 230)	0.964 (0.875 – 0.996)	0.983 (0.957 – 0.995)	0.930 (0.830 – 0.981)	0.991 (0.969 – 0.999)
91 – 180 days (I: 5; NI: 33)	1.000 (0.478 – 1.000)	1.000 (0.894 – 1.000)	1.000 (0.478 – 1.000)	1.000 (0.894 – 1.000)
>180 days (I: 0; NI: 0)	-	-	-	-
Overall (I: 61; NI: 421)	0.967 (0.887 – 0.996)	0.986 (0.969 – 0.995)	0.908 (0.810 – 0.965)	0.995 (0.983 – 0.999)

Conclusions (1)

- Up24 showed good overall sensitivity (95.8%) and specificity (98.7%) as compared to known HIV-1 infection status using the standard extraction buffer protocol.
- Using the new extraction buffer protocol there was no difference in specificity with a slight increase in sensitivity (96.7%) although whether this is significant could not be determined with the limited numbers tested.
- While specificity was always good, sensitivity was excellent after one week from birth using either buffer system.

Conclusions (2)

- Time to detection could not be accurately determined as there were very few <30 day samples but good sensitivity was seen with both buffer systems >30 days after birth.
- Up24 may be useful as an inexpensive method to diagnose HIV-1 infection in infants in resource-limited settings after more evaluations.

Future Studies

- Additional studies are needed to determine the need for the new lysis buffer as it is not provided with the current assay configuration.
- Evaluations from infected infants at time points closer to birth are also needed to determine usefulness for early diagnosis.
- CDC-Global AIDS Program has initiated targeted evaluations of Nucleic Acid and Up24 assay focusing on Dried Blood Spots in diverse geographic locations (Africa, Caribbean) to better determine their performance and possible application in these resource-limited settings.

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