

Ultrasensitive p24 Assay for Infant HIV Testing in Lilongwe, Malawi

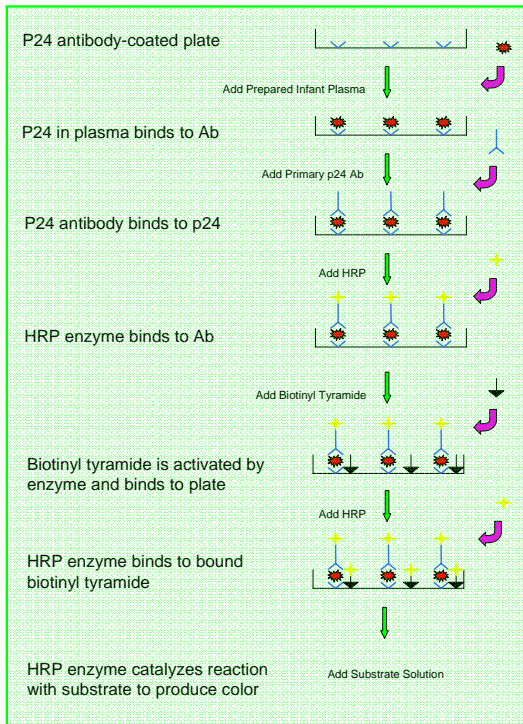
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Background: Nearly all of the 540,000 children infected with HIV in 2005 were infected at or near the time of birth. Standard antibody tests cannot be used to diagnose these infants because maternal HIV antibodies cross the placenta and can remain in the infant circulation up to 1 year of age. To test infants clinicians must rely on expensive and technologically complex nucleic acid assays that detect the virus itself. In the past standard acid or base dissociated p24 antigen assays were proven to be too insensitive for infant diagnosis. The PerkinElmer Life and Analytical Sciences Ultrasensitive p24 Antigen assay (Up24 assay), optimized by Dr. Jorg Schupbach, is now available and includes a signal amplification step. This is a significantly cheaper and simpler alternative to DNA PCR and uses the familiar technology of antibody tests. The Up24 test has shown >95% sensitivity and specificity in prior studies, some of which took place in developing countries. In 2005, the Up24 assay was performed in Lilongwe, Malawi using infant plasma samples from the UNC Project Breastfeeding, Antiretroviral, and Nutrition (BAN) study. When these results were compared to the gold standard DNA PCR results, a large number of false positives caused specificity to be <50%.

Methods: After training at UNC in Chapel Hill, NC, by an experienced researcher, I went to the UNC Project in Lilongwe, Malawi for 5 weeks to perform the Up24 assay. A Malawian technician was trained to perform the Up24 assay. Plasma collected from infants during the BAN study at various ages (birth- 7 months), that had been previously tested by DNA PCR, was used.

Figure 1: Flow- Chart of Ultrasensitive p24 Assay



Results: In the initial test of the Up24 assay at the UNC Project in Malawi showed 100% sensitivity as compared to DNA PCR, yet only 44% specificity. This high rate of false positive results was not seen with previous use of the Up24 assay in Chapel Hill, thus a second round of testing was completed in Chapel Hill with similar samples. In this second round sensitivity was reduced to 50%, yet specificity increased to 99%. This study was at the UNC Project and performed with similar results those from Chapel Hill with sensitivity and specificity at 55 and 99%, respectively.

Table 1: 2 x 2 Table of Up24 Assay Results

	DNA Positive	DNA Negative
P24 Positive	6	2
P24 Negative	5	227

Table 2: Results of Up24 Test Performed on Malawian Samples

	Sensitivity	Specificity	PPV	NPV
Malawi- 1 st Attempt	100	44	2	100
Malawi Samples in Chapel Hill, NC	50	99	75	96
Malawi- 2 nd Attempt	55	99	75	98

Conclusions: Earlier problems with false positive results did not occur. Sensitivity and specificity results from the assay performed in Malawi were similar to those in Chapel Hill, NC, demonstrating no inherent problem of implementing the Up24 assay in the UNC Project lab in Lilongwe, Malawi. There was, however, poor sensitivity of the assay. This could be a result of the small number of positives in the samples tested, or the antiretroviral drugs given to the infants in this study. These possibilities will be investigated by future testing of samples from Malawian infants who did not receive drugs and with higher HIV prevalence in the test infant samples.

Acknowledgements:

