

## HIV-1 Diagnosis of Infants Using Dried Blood Spots and the Ultra-sensitive p24 Antigen Assay

<i>Abstract Category:</i>	New HIV Diagnostic Technologies Including Those That Are Not FDA Approved
<i>Primary Author:</i>	Ada Cachafeiro
<i>Affiliation:</i>	University of North Carolina at Chapel Hill
<i>Co-Authors:</i>	Gayle Sherman, Annette H. Sohn, Consuelo Beck-Sague, Susan A. Fiscus

### OBJECTIVE

To optimize the ultrasensitive p24 antigen (Up24 Ag) assay to work with dried blood spots (DBS) for the diagnosis of HIV infection in infants.

### METHODS

DBS were collected on Whatman 903 paper, air dried, stored with a desiccant in individual ziplock bags at room temperature or at 4°C (Vietnamese) and shipped to UNC. The final optimized procedure used a modified elution buffer containing TritonX100 and Tris found in the Perkin Elmer Up24 Ag kit and an enhanced specimen diluent. After adding the enhanced diluent and Triton/Tris/PBS buffer to two 6mm DBS punches, the specimens were rocked at room temperature for 2 hours or overnight at 4°C. The package insert was followed thereafter, except that positive and negative controls also consisted of DBS. Results from the Up24 antigen assay were compared with infant diagnoses by DNA PCR for specimens (US, Vietnam and South Africa), or with RNA viral load results (Malawi and the Dominican Republic).

### RESULTS

A total of 411 DBS from HIV exposed infants were tested: 227 presumably subtype B from the US (167) and Dominican Republic (60), 109 presumably subtype C from Malawi (9) and South Africa (100), and 75 presumably subtype A/E from Vietnam. Overall sensitivity and specificity were 86.7 % and 100%, respectively. The 10 false negative specimens were from infants receiving antiretrovirals for prophylaxis (n=3) or treatment (n=7). When these were excluded from the analysis, the sensitivity and negative predictive value increased to 100%. DBS stored at room temperature for as long as 18 months still gave reliable results.

All specimens N=411	Sensitivity 86.7% Specificity 100% PPV 100% NPV 97.1%	US/Dominican Republic N=227	Sensitivity 93.8% Specificity 100% PPV 100% NPV 99.5%
Malawi/South Africa N=109	Sensitivity 84.1% Specificity 100% PPV 100% NPV 90.3%	Vietnam N= 75	Sensitivity 86.7% Specificity 100% PPV 100% NPV 96.7%

### CONCLUSIONS

The Up24 Ag assay was modified for use with DBS for the diagnosis of vertical HIV infection. DBS stored up to 18 months gave reliable results and subtypes B, C, and A/E were consistently detected. Infected infants who are receiving antiretrovirals may not always be detected in this assay.