Abstract 10

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

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Introduction

Diagnosis of HIV infection in infants is hindered by the transfer of maternal immunoglobulins across the placenta. As a consequence, antibody tests including the simple, inexpensive, and widely used rapid antibody tests, cannot be used. Instead, technologically more complex and expensive nucleic acid assays are typically used. Dried blood spots (DBS) have been shown to work well in HIV RNA and DNA assays for the diagnosis of infant infection (1,2).

The ultra-sensitive p24 antigen assay is an alternative test that overcomes the barrier to detection caused by maternal antibody, while using an ELISA-based platform that reduces the need for expensive laboratory equipment and complex infrastructure. Earlier attempts to adapt the Perkin Elmer p24 antigen assay had mixed results (3-5) and sometimes employed a complicated buffer (4-5). We sought to develop a simpler method using reagents that are already part of the commercially-available Up24 test kits (PerkinElmer Life Sciences).

Methods

DBS were collected on Whatman 903 paper, air dried, stored with a dessicant 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-HCl found in the Perkin Elmer Up24 Ag kit and a Specimen Preparation Diluent. After adding the Specimen Preparation Diluent and Triton/Tris/PBS buffer to two 6mm DBS punches, the specimens were shaken 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).

Table 1. Comparison of different extraction methods used for p24 antigen assays with DBS. Reagents in red are not included in the P-E kit.

Li, et al 2005 (3)	*Knuchel, et al 2007 (4)*	*Patton, et al 2006 (5)*	*Cachafeiro, et al*
Elute 50 ul DBS using 0.5% Triton X100 incubated 60 min. Centrifuge 13,6000 x g for 3 min. Transfer to screwcap cryovial and boil for 5 min at 100°C. Proceed with the Up24 antigen assay.	16 mm DBS plus 0.8 ml of elution buffer (1 part SNCR* buffer, 1 part water, 9 parts 0.5% Triton X100). Incubate 8 hr at room temp with shaking. Boil for 5 min at 100°C. Proceed with the Up24 antigen assay with modifications. After the first wash quench with 0.3% H_2O_2 in 50mM Tris-HCl for 10 min at 37°C. *SNCR buffer = 30mM Tris-HCl (pH 7.2), 450mM NaCl, 1.5% Triton X100, 1.5% deoxycholic acid, 0.3% SDS, 10mM EDTA.	Duplicate 6mm punches incubated with 25 ul SNCR* buffer 10 min at room temp. Add 275 ul 0.5%Triton X100. Incubate overnight at 4°C. Boil for 5 min at 100°C. Proceed with the Up24 antigen assay. *SNCR buffer = 30mM Tris-HCl (pH 7.2), 450mM NaCl, 1.5% Triton X100, 1.5% deoxycholic acid, 0.3% SDS, 10mM EDTA.	Duplicate 6 mm punches incubated with 50 ul Specimen Preparation Buffer** 10 min at room temp. Add 250 ul of 9 parts 0.5% Triton X100, 1 part Tris HCl. Shake for 2 hr at room temp or overnight at 4 C. Boil for 5 min at 100°C. Proceed with the Up24 antigen assay. **Specimen Preparation Buffer = Kit specimen diluent, 10mM EDTA, 30mM Tris-Hcl (pH 7.2)

Results

Table 2. Sensitivity and Specificity of the Up24 antigen assay by country of origin. US and Dominican Republic – presumed subtype B, South Africa and Malawi – presumed subtype C, and Vietnam – presumed subtype AE.

	All Specimens		Specimens Except those on ART	
Specimens	Sensitivity (+/tested)	Specificity (+/tested)	Sensitivity (+/tested)	Specificity (+/tested)
United States (N=167)	3/4 (75%)	163/163 (100%)	3/3 (100%)	163/163 (100%)
Dominican Republic (N=60)	12/12 (100%)	48/48 (100%)	12/12 (100%)	48/48 (100%)
South Africa (N=100)	30/35 (86%)	65/65 (100%)	30/31 (97%)	65/65 (100%)
Malawi (N=9)	7/9 (78%)	None tested	7/7 (100%)	None tested
Vietnam (N=79)	15/19 (79%)	60/60 (100%)	12/12 (100%)	60/60 (100%)
Total (N=415)	67/79 (85%)	336/336 (100%)	63/64 (98%)	336/336 (100%)

Table 3. Sensitivity and specificity of the Up24 antigen assay with DBS by age

Age	Sensitivity	Specificity
<7 days N=60	2/5 (40%)	55/55 (100%)
1-6 weeks N=64	4/4 (100%)	60/60 (100%)
6-26 week N=136	19/19 (100%)	118/118 (100%)
>26 weeks N=127	40/49 (82%)	18/18 (100%)

Results

Table 4. Characteristics of the 12 false negative specimens. 11 of 12 were from patients on ARVs either for treatment or prophylaxis.

Subject	Country	Temp	ARVs	Age	VL
01576	US	15 day -20°C	AZT	3 days	ND
0960	Malawi	Ambient 15 mo	sdNVP + CBV	1 day	ND
1850	Malawi	Ambient 8 mo	sdNVP+CBV	1 day	ND
235G	Vietnam	4-8°C 3 mo	On ART	18 mo	53,600
428D	Vietnam	4-8°C 3 mo	On ART	9 mo	<250
332F	Vietnam	4-8°C 2 mo	On ART	18 mo	1880
308G	Vietnam	4-8°C 2 mo	On ART	12 mo	13,000
A018	South Africa	Ambient 18 mo	On ART	14 mo	19,000
A051	South Africa	Ambient 14 mo	On ART	9 mo	<25
A053	South Africa	Ambient 13 mo	No longer on ART	6 yr	66,000
A061	South Africa	Ambient 13 mo	On ART	17 mo	290,000
A079	South Africa	Ambient 13 mo	On ART	5 yr	70

Conclusions

1. HIV-1 infection in infants can be accurately diagnosed using DBS and the Up24 antigen assay.
2. Anti-retrovirals used for prophylaxis or treatment can sometimes lead to false negative results.
3. The assay offers a lower cost, technologically less complex method for early infant diagnosis.

References

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