

# HIV-specific IgA ELISA: A Cost-effective Diagnostic Tool and Progression Marker for HIV Infection in Vertically Exposed Children in Developing Countries like India

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Abstract

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## Abstract

**Background:** Diagnosis of HIV infection in infants born to HIV-infected mothers is problematic in many resource-poor countries. Conventional HIV IgG ELISA cannot be used due to presence of passively transferred maternal HIV IgG antibodies and is useful only after 18 months of age. Availability of HIV DNA PCR is limited because of technical and financial constraints. In search of a low-cost diagnostic tool we evaluated HIV-specific IgA assay by ELISA, which is available in most laboratories in India.

**Methods:** In this study, vertically exposed infants (n = 150) born to HIV+ mothers were recruited: group I, neonates < 28 days (n = 50); group II, > 28 days to < 18 months (n = 50); group III, > 18 months (n = 50). Patients in group I were followed prospectively. ELISA was standardized for detection of HIV specific IgA antibodies to HIV-1 and -2 antigens in serum after absorption of IgG antibodies. The results of HIV specific IgA ELISA were compared with HIV DNA PCR and p24 antigen assay. PCR for detection of HIV1 DNA was performed using gag and env primers and LTR-specific nested primers were used for detection of HIV 2. p24 antigen detection was performed with a commercial kit.

**Results:** In the 150 children recruited in this study, HIV transmission had occurred in 48% (73 of 150) of children as determined by HIV DNA PCR. HIV 1 was the predominant infection, with only 1 case of HIV2. HIV-specific IgA ELISA was evaluated taking HIV DNA PCR as the gold standard. HIV-specific IgA ELISA was negative in all neonates < 28 days of age. On prospective follow up of these infants, HIV-specific IgA antibody appeared from 3 to 9 months of age. In infants < 18 months, the sensitivity of HIV specific ELISA was found to be 71% and specificity was 100%. The sensitivity of p24 antigen ELISA was 42% and specificity was 100%. In infants > 18 months the test correlated well with the disease severity. HIV specific antibody was found in 31% asymptomatic vs 100% symptomatic cases. Presence of p24 antigen showed 100% concordance with HIV specific IgA antibodies in symptomatic HIV-infected cases of this group.

**Conclusions:** HIV specific IgA ELISA can be used for diagnosis of vertically transmitted HIV infection in infants less than 18 months of age in resource poor countries. The presence of HIV specific IgA antibodies in cases with symptomatic disease could reflect extreme B-cell activation. The utility of this assay as a marker of disease progression needs further investigation.

## Introduction

In India, though the major mode of transmission is heterosexual, the infection is slowly spreading to low risk behavior groups represented by HIV donors and antenatal women. Vertical transmission from an infected mother to her child occurs either in utero, during labor and delivery or postpartum through breastfeeding. In Mumbai the prevalence in antenatal women is reported to be 3%. As the number of women infected with HIV increases, the number of HIV infected children born to these seropositive mothers also increases.

Diagnosis of HIV infection in infants born to HIV-infected mothers is problematic in many resource-poor countries. Conventional HIV IgG ELISA cannot be used due to presence of passively transferred maternal HIV IgG antibodies and is useful only after 18 months of age. Availability of HIV DNA PCR is limited because of technical and financial constraints. In search of a low-cost diagnostic tool we evaluated HIV-specific IgA assay by ELISA, which is available in most laboratories in India.

## Objectives

- To standardize HIV specific IgA ELISA -a cost effective test that can be done in available ELISA setting.
- To use HIV specific IgA ELISA for diagnosis of HIV infection in vertically exposed neonates and children.
- Comparative evaluation of HIV-specific IgA ELISA with HIV DNA PCR and p24 antigen assay.
- Correlation of HIV specific IgA results to infants clinical state.

## Materials and Methods

### CONTROL GROUPS n = 100

- A) Known HIV positive individuals n = 50  
B) HIV seronegative healthy individual n = 50

### TEST GROUP : Vertically exposed infants n = 150

- I) Infants born to HIV positive mothers <28 days n = 50  
II) Infants born to HIV positive mother >28 day <18 months of age n = 50  
III) Children born to HIV seropositive mothers >18 months n =50

### HIV specific IgA ELISA

#### Removal of IgG using Protein A

Microtitre plates were coated with Protein A (1ug/well)  
Serum samples were added to the plate - incubate 37 c -45mins  
(Shaker incubator) and transferred to Antigen coated wells.

#### HIV IgA ELISA was standardized

checked board titration method.  
Antigens: microtitre plates coated with gp120, gp41 and p24 for HIV1 and gp36 for HIV 2  
Serum dilution of 1:10 and anti IgA HRP conjugate dilution of 1:100  
Substrate: OPD peroxidase.

#### Calculation of the Cut-off value

100 healthy individuals were used to calculate Standard Deviations.  
Cut off was calculated by adding three Standard Deviations [i.e. 3 X 0.0105 = 0.0315] to the mean of negative control.  
Cut off = Mean of negative control + 0.0315.

### p24 antigen ELISA

P 24 antigen ELISA for detection of p24 core antigens of HIV-1 subtype O & HIV2 was performed using commercially available kit from Innogenetics, Belgium.

**Enzyme:** Horse Radish Peroxidase. **Conjugate:** anti p24 monoclonal.

**Substrate:** Tetramethyl benzidine TMB / Hydrogen peroxidase.

### HIV DNA PCR

**Extraction of DNA:** Extraction by direct lysis of PBMCs

**Amplification of DNA:**

1 µg of genomic DNA was amplified  
*HIV 1 DNA PCR using gag and env primers.*

Master mix:Primers:50 p moles, dNTPs 200µM, Taq 2units, Mgcl2 1.5mM  
gag primers SK38 ATATTCACCTATCCAGTAGGAGAAAAT  
SK39 TTGGTCTGTCTATTCCAGAAATGC  
env primers env1 ATAGCTCAATGTACACATGGAATT  
env2 ATTACAGTAGAAAAATCCCC

*HIV 2 DNA PCR using Nested primers.*

Master mix:Primers:50 pmoles, dNTPs 200µM, Taq 2units, Mgcl2 6 mM.  
LTR 0G01 AGAGGCTGGCAGATTCGACCCCTGGC  
0G24 AAGGGTCTAACAGCCAGGT  
0G04 TCCAGCACTAGCAGGTAGCAGCCGT  
0G19 GACCAGGGCCGACTAGGAGAAGATG

**Visualization of Amplified Product:**

Visualized by Ethidium Bromide (0.5ug/ml) staining of 2% Agarose gel under U.V. transilluminator.

**Results:**

*HIV 1 DNA PCR (gag) Positive : 115 base pair band*  
*HIV1 DNA PCR (env) Positive : 422 base pair band*  
*HIV2 DNA PCR Positive: 168 base pair band*

### Tests for detection of HIV 1 & 2 IgG antibody ELISA

#### Screening test:

A third generation HIV antibody ELISA was used.  
HIV EIA that detects IgG antibodies to HIV-1 gp120, gp41 and p24 and HIV-2 gp36 in human serum was used.

**Enzyme:** HRP, **Substrate:** TMB / Hydrogen peroxidase

**Confirmatory Test :** Line Immuno assay for HIV1&2 ( Immunogenetics)

**Enzyme:** Alkaline phosphatase, **Substrate:** (BCIP) and nitroblue tetrazolium (NBT)

## Results

**Table 1.** DNA PCR results in known HIV positive and known HIV negative samples of control group A and group B

category	Sample No.	HIV 1 DNA PCR gag	HIV 1 DNA PCR env	HIV 2 DNA PCR LTR
Known HIV+ ( n=50)	HIV 1 (n=30)	30 (100%)	29 (96%)	0
	HIV 2 (n=8)	0	0	8 (100%)
	HIV 1 & 2 (n=12)	12 (100%)	10 (83%)	12
Known HIV- ( n=50)	n=50	0	0	0

HIV DNA PCR using gag primers was more sensitive than env primers.

**Table 2.** Diagnosis of HIV infection in vertically exposed children using PCR

Groups	HIV infected	Uninfected	Total
< 28 days	22 (44%)	28	50
<18 months	20 (40%)	30	50
>18 months	31 (62%)	19	50
Total	73 (48%)	77	150

HIV transmission occurred in 48% of vertically exposed children.

**Table 3.** Diagnosis of HIV 1 & 2 infection in vertically exposed children using PCR

Groups	HIV 1 DNA PCR: gag	HIV 1 DNA PCR: env	HIV2 DNA PCR: LTR
Neonates <28 days	22	20	0
Infants <18 mo	20	18	0
Children >18 mo	30	28	1
Total: 73	72	66	1

HIV-1 was predominant infection and only one case was HIV2 was detected.

## Results

**Table 4.** Appearance of HIV IgA antibodies in HIV infected neonates.

	1 <sup>st</sup> visit at < 28 days	2 <sup>nd</sup> visit by 3-9 months of age
HIV IgA + ve	0	17 (77.2%)
HIV IgA - ve	22	5 (22.7%)
Total	22	22

HIV specific IgA antibodies appeared from 3 to 9 months of age.

**Table 6.** Sensitivity & Specificity of HIV IgA ELISA in children < 18 months.

	HIV DNA PCR POSITIVE	HIV DNA PCR NEGATIVE
HIV IgA +VE	30 (71%)	0
HIV IgA -VE	12 (28%)	58 (100%)
TOTAL	42	58

In children <18 months the sensitivity of HIV IgA was 71% and Specificity was 100 % The subjects are group I follow up infants and that of group II.

**Table 8.** Correlation of HIV IgA ELISA with clinical stage of HIV disease infant > 18 months.

	Asymptomatic	Symptomatic
HIV IgA +VE	6 (31%)	12 (100%)
HIV IgA -VE	13	0
Total	19	12

HIV IgA ELISA correlated well with the disease severity. All symptomatic infants >18 months of age had HIV IgA antibodies.

**Table 5.** Appearance of HIV IgG antibodies in HIV infected neonates.

Age (months)*	Group I <28 days	Group II >28 days<18mo	TOTAL
7-9 months	9 (32%)	5 (16.6%)	14 (24%)
10-12months	12 (42.8%)	21 (70%)	33 (56.8%)
13-15 months	4 (14%)	1 (3.3%)	5 (8.6%)
16-18 months	3 (10.7%)	3 (10%)	6 (10.3%)
Total	28 (48%)	30 (51.7%)	58

Anti HIV IgG appeared by 15 months of age.

**Table 7.** Sensitivity & Specificity of P24 antigen ELISA in children < 18 months.

	HIV DNA PCR POSITIVE	HIV DNA PCR NEGATIVE
P 24 ANTIGEN +VE	18 (42%)	0
P 24 ANTIGEN -VE	24	58 (100%)
TOTAL	42	58

The sensitivity of p24 antigen ELISA was 42% and specificity was 100%.

**Table 9.** Correlation of p24 Antigen ELISA with HIV IgA ELISA in symptomatic children > 18 months.

	P24 antigen +ve	P24 antigen -ve	Total
HIV IgA +VE	11 (100%)	1	12
HIV IgA -VE	0	0	0
Total	11	1	12

Presence of p24 antigen showed 100% concordance with HIV specific IgA antibodies in symptomatic HIV infected children >18 months.

## Conclusions

HIV IgA appeared from 3-9 months of age. The sensitivity of HIV IgA ELISA v/s p24 antigen ELISA was 71% v/s 42% and specificity of both assays was 100% in infants <18 months of age. Since it can detect antibodies as early as 3 month and is sensitive as compared to p24 antigen, HIV IgA ELISA can be used as a diagnostic test for diagnosis of HIV infection in vertically exposed infants where PCR facilities are not available.

HIV specific IgA antibody was present in 100% symptomatic infants and in 31% asymptomatic infants >18months. There was 100% concordance with p24 antigen ELISA in symptomatic cases. The presence of HIV specific IgA antibodies could reflect extreme B cell activation. Its utility as a marker of disease progression can be further assessed.

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