

## Original Research Article

# A comparative study on fourth generation ELISA and rapid assay for diagnosing HIV infection in the high risk group

Sukanya Mathupal<sup>1</sup>, Sathya Narayanan Rajendran<sup>2\*</sup>

Department of Skin and STD, <sup>1</sup>Sree Balaji Medical College Hospital, <sup>2</sup>Saveetha Medical College Hospital, Chennai, Tamil Nadu, India

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### \*Correspondence:

Dr. Sathya Narayanan Rajendran,

E-mail: [sathya.vinukonda@gmail.com](mailto:sathya.vinukonda@gmail.com)

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## ABSTRACT

**Background:** Enzyme linked immunosorbent assay (ELISA) is a widely employed method for detecting HIV. Following its successful detection of HIV, the fourth generation ELISA was approved by FDA. The advantage of the FDA approved fourth generation ELISA is it detects p24 antigen, HIV 1&2 antibodies simultaneously and that too 5-7 days prior to conventional tests so that it detects HIV seroconversion earlier reducing its further spread.

**Methods:** The study was conducted in a tertiary level medical centre after obtaining ethical approval. 200 patients from high risk group and their contacts were selected after a thorough physical examination and tests were done using fourth generation ELISA and rapid assay and these results were compared.

**Results:** As per our observations, the percentage of patients tested positive by fourth generation ELISA out of 200 subjects was 37% and negative was 63%. The detection rate of HIV positivity by rapid assay in high risk group was 11% and negative was 89%. The detection rate was also higher with fourth generation ELISA when various other parameters were compared.

**Conclusions:** In this study, the percentage of HIV positivity detected by fourth generation ELISA was higher than that detected by rapid assay showing that fourth generation ELISA is a more sensitive test than rapid assay as well as a better diagnostic modality to opt for when coming to early detection in HIV.

**Keywords:** p24 antigen, Fourth generation ELISA, High risk group

## INTRODUCTION

Enzyme linked immunosorbent assay (ELISA) is a widely employed method for detecting HIV. Many samples are tested at once and results are obtained within few hours. Following the successful detection of HIV by ELISA, the fourth generation ELISA was approved by FDA. The main advantage of fourth generation ELISA is reduction of the window period due to screening for p24 antigen as well as anti HIV 1 and anti HIV 2 simultaneously. The additional advantage of detecting p24 Ag helps the latest fourth-generation ELISA to pick

up infections 5 to 7 days in advance.<sup>1</sup> The total window period can be brought down to two weeks using p24-antigen assay.<sup>2</sup> This reduction of window period and early detection of HIV seroconversion helps in reduction of further spread of the disease by counselling the patients regarding abstinence, barrier contraception, avoidance of needle sharing and partner screening.

This study is done to detect HIV infection in high risk category using fourth generation enzyme linked immunosorbent assay and to compare the results of test 1 (fourth generation ELISA) with test 2 (rapid assay).

## METHODS

This prospective observational study was conducted in Sree Balaji Medical College hospital between August and December 2015.

### Inclusion criteria

200 patients were selected by simple random sampling from the high-risk group and their contacts which included commercial sex workers, men with recent or recurrent exposure to commercial sex workers, females whose husbands were promiscuous, transgenders, men seeking men, partners of HIV seropositive patients, partners of VDRL reactive patients, victims of sexual abuse, intravenous drug abusers, patients who had multiple blood transfusions and occupational exposure to blood products. Complete history taking and thorough physical examination was done in all patients. Written informed consent was obtained.

### Exclusion criteria

Known HIV positive patients and patients less than 10 years were excluded from the study. After explaining the procedures patients who refuse to participate in the study were also excluded.

After fulfillment of the above inclusion criteria, patients were shortlisted and enrolled in the study. Complete history taking and thorough physical examination was done in all patients. After counselling and after recording their consent for the test, fourth generation ELISA for HIV infection was conducted on these selected patients along with routinely done rapid assay for HIV infection. 5 ml blood was withdrawn aseptically from the patient. The serum was separated and subjected to Fourth Generation ELISA as well as Rapid Assay for detection of HIV infection

### Study principle

**Test 1:** It is a generation four solid phase enzyme linked immuno sorbent assay based on the principle of double antigen/antibody sandwich technique for the detection of IgM and IgG antibodies against HIV 1 and/or HIV 2 and p24 antigen in human serum or plasma. The kit used in this study employs monoclonal antibodies to p24 and highly purified antigens (recombinant) like gp41 (envelope glycoprotein) of HIV 1 and gp 36 (envelope glycoprotein) of HIV 2.<sup>3</sup>

**Test 2:** Dot ImmunoAssay employs the same principle as enzyme immuno assay whereby the immobilized antigen antibody complex is visualized by means of colour producing (chromogenic) reaction. They are based on immunoconcentration method. HIV antigens both of HIV 1 and 2 are impregnated on comb shaped solid support. Sample used is diluted in specimen diluent and then added to the kit. Patient's sera containing HIV antibody

binds to the antigen in the kit and get immobilized. Protein A colloidal gold conjugate which is the signal reagent is then added to the kit. This protein A attaches to the Fc portion of anti-HIV antibodies. The coloured end point is developed by a colloidal gold protein - a signal reagent. A reddish spot proves the formation of immune complex, which further confirms the presence of anti-HIV antibodies in the test serum.

Using Chi Square test, the results of both fourth generation ELISA as well as rapid assay would be compared.

## RESULTS

As per our observations, the percentage of patients tested positive by fourth generation ELISA out of the 200 patients were 37% and negative were 63%. The detection rate of HIV positivity by rapid Assay in the study group was 11% and 89% tested negative.

**Table 1: Prevalence of HIV among the various genders in the study population by fourth generation ELISA.**

Gender	Negative N (%)	Positive N (%)	Total N (%)
Female	19 (63.3)	11 (36.7)	30 (100)
Male	97 (61.8)	60 (38.2)	157 (100)
Transgender	10 (76.9)	3 (23.1)	13 (100)
Total	126 (63)	74 (37)	200 (100)

**Table 2: Prevalence of HIV among the various genders in the study population by rapid assay.**

Gender	Negative N (%)	Positive N (%)	Total N (%)
Female	26 (86.7)	4 (13.3)	30 (100)
Male	141 (89.8)	16 (10.2)	157 (100)
Transgender	11 (84.6)	2 (15.4)	13 (100)
Total	178 (89)	22 (11)	200 (100)

In our study, the maximum populations were men constituting 157 in number of whom 38.2% were tested positive by fourth generation ELISA. Whereas, among 30 females, 36.7% and among the 13 transgenders, 23.1% of them tested positive by fourth generation ELISA (Table 1). By rapid assay, among the 157 males, 10.2% (16 patients) were HIV positive. 13.3% (4 patients) among the 30 females and 15.4% (2 patients) among the 13 Transgenders were also HIV Positive by Rapid Assay (Table 2). The p value was 0.047 which is statistically significant.

The maximum tested Positive for HIV by fourth generation ELISA was in 31-40 years category (52.1%) (Table 3). A similar pattern was observed in Rapid Assay (Table 4). The majority of patients who can otherwise be termed as MARG (most at risk group) were those

exposed to CSWs which contributed to 65.5% (131 patients) of the study group. The next highest group was those, whose partners had multiple exposure to CSWs. (Table 5). There was almost an equal distribution between transgenders and MSMs among the homosexuals. There were obvious overlap of categories, like bisexuals or transgenders or those whose spouse were exposed to commercial sex workers and sexually transmitted infections and considerable number of patients had multiple risk factors.

**Table 3: Prevalence of HIV among various age groups by fourth generation ELISA.**

Age in years	Negative N (%)	Positive N (%)	Total N (%)
<20	13 (76.5)	4 (23.5)	17 (100)
21–30	49 (62.8)	29 (37.2)	78 (100)
31–40	23 (47.9)	25 (52.1)	48 (100)
>40	41 (71.9)	16 (28.1)	57 (100)
<b>Total</b>	<b>126 (63)</b>	<b>74 (37)</b>	<b>200 (100)</b>

**Table 4: Prevalence of HIV among various age groups by rapid assay.**

Age in years	Negative N (%)	Positive N (%)	Total N (%)
<20	17 (100)	0 (0)	17 (100)
21–30	70 (89.7)	8 (10.3)	78 (100)
31–40	39 (81.3)	9 (18.7)	48 (100)
>40	52 (91.2)	5 (8.8)	57 (100)
<b>Total</b>	<b>178 (89)</b>	<b>22 (11)</b>	<b>100 (100)</b>

**Table 5: High risk categorization in the study group.**

High risk category	Number	Percentage (%)
CSW	9	4.5
Sexual abuse	1	0.5
Homosexuals	14	7
Bisexuals	10	0.5
Transgender	13	6.5
Exposure to CSW	131	65.5
Spouse exposed to CSW	15	7.5
Contact tracing	7	3.5

**Table 6: HIV detection by fourth generation ELISA in patients with complaints.**

Complaints	Negative N (%)	Positive N (%)	Total N (%)
Nil	66 (65.3)	35 (34.7)	101 (100)
Genital	48 (59.3)	33 (40.7)	81 (100)
Non specific	10 (62.5)	6 (37.5)	16 (100)
Partner on art	1 (100)	0 (0)	1 (100)
Sexual abuse	1 (100)	0 (0)	1 (100)

**Table 7: HIV detection by rapid assay in patients with complaints.**

Complaints	Negative N (%)	Positive N (%)	Total N
Nil	91 (90.9)	10 (9.1)	101
Genital	72 (88.9)	9 (11.1)	81
Non-specific	13 (81.3)	3 (18.8)	16
Partner on art	1 (100)	0 (0)	1
Sexual abuse	1 (100)	0 (0)	1

The majority of patients 50.5% (101 patients) came without any complaints to our STD out-patient department. Of which 34.7% (35 patients) were tested positive for HIV by fourth generation ELISA. Out of these 81 patients who presented with genital complaints, 40.7% (33 patients) were HIV positive. Out of the 16 patients who presented with non-specific symptoms, 6 were HIV positive making it the second maximum percentage of HIV positivity (Table 6).

Whereas rapid assay was able to detect HIV positivity in 18.8% (3 patients) of patients with nonspecific symptoms and 11.1% (9 patients) among the patients with genital complaints. Interestingly, HIV positivity was detected in 9.9% (10 patients) in the sector of patients who presented without any complaints, but with only recent exposure to commercial sex worker (Table 7). In rapid assay, the maximum HIV detection was seen in people with non-specific symptoms.

## DISCUSSION

Human immunodeficiency virus (HIV) is a lentivirus (slowly replicating retrovirus) that causes acquired immuno deficiency syndrome (AIDS), a condition which causes progressive failure of the immune system allowing life-threatening opportunistic infections and cancers to thrive. AIDS was postulated initially as an infective condition way back in 1981 by Gottlieb et al.<sup>4</sup> Within a span of four decades, it has established to become a global pandemic imposing a major threat to different walks of life. It has immense effect on social as well as economic perspectives of nations.

The screening tests employed in detection of HIV are ELISA and rapid assays. ELISA is a widely employed method for detecting HIV. The fundamental principle of ELISA is to utilize an enzyme to detect the antigen antibody complex formation. There are four generations of ELISA of which fourth generation is the latest combo assay. This help in identifying cases earlier shortening the window period.<sup>5,6</sup>

The positivity in transgenders in our study is in contrast to Setia et al who studied focussing only transgenders and MSMs involved in commercial sex where 68% of transgenders were tested positive for HIV by rapid assay and ELISA.<sup>7</sup>

The age-based HIV detection rate in our study shows a maximum positivity in 31-40 years. This is in concordance with the observations by Olufemi et al where the maximum HIV positive patients were in their second and third decade (age group).<sup>8</sup> The detection of HIV was highest for the category of patients who presented with genital symptoms and second highest in those with non-specific symptoms by fourth generation ELISA. This was in contrary to a Nigerian study by

Olufemi et al with a similar or comparable study of 218 patients where the foremost presentation observed was oral thrush.<sup>8</sup> In our study, only one case presented with oral candidiasis.

## CONCLUSION

In this study, the percentage of HIV positivity detected by fourth generation ELISA was higher than that detected by rapid assay showing that fourth generation ELISA is a more sensitive test than rapid assay. 20-40 years seems to be the age group with maximum number of seropositivity detected. Fourth generation ELISA helps in earlier detection of HIV and hence controls further spread of infection. The current trend is to pick early and hit early. Patients can be started on antiretroviral treatment at the earliest bringing down his viral load and complications. Early initiation of retroviral therapy lessens the incidence of opportunistic infections as well as AIDS related co morbidities and death.

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