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Medicine

COMPARISON OF HIV FOURTH GENERATION RAPID TEST WITH HIV FOURTH GENERATION ELISA FOR SCREENING VOLUNTARY BLOOD DONORS.

Dr Satish Kumar	AFTC, Delhi Cantt-110010
Dr Rajat Jagani	AFTC, Delhi Cantt-110010
Dr Amit Pawar*	AFTC, Delhi Cantt-110010 *Corresponding Author
Dr Ujjwal Dimri	AFTC, Delhi Cantt-110010

ABSTRACT

Introduction: Fourth-generation assays permit the simultaneous detection of HIV antigen and antibody. With this test, it is possible to detect HIV p24 antigen during the window period, if the blood donor happens to be tested during the short peak of high levels of circulating virus particles.

Material and Methods: In this prospective study, we screened a total of 1599 blood donors which included both whole blood donors and apheresis donors. All samples were screened with 4th generation ELISA (RFCL Limited), 4th generation Rapid Immunochromatograpy test (TRI-DOT+Ag, J. Mitra. & Co. Pvt. Ltd) and real time PCR technique.

Result and Discussion: In this study, four positive samples were detected by 4th generation ELISA, out of which two samples showed marginal positivity. All four samples were negative by 4th generation Rapid TRI-DOT +Ag, but positive with Real Time Polymerase Chain Reaction (RT-PCR) thereby implying a sensitivity of 4th gen ELISA comparable to RT-PCR, whereas sensitivity of 4th gen Rapid TRI-DOT was greatly inferior to both 4th gen ELISA and RT-PCR because of the inherent inability of the 4th gen RAPID to pick up cases of low antigenemia and low antibody titer

Conclusion: 4th generation ELISA should be continued for blood donor screening and rapid TRI-DOT should not be used as an alternative to 4th generation ELISA. For a resource poor developing country like India, till the country is able to implement NAT/PCR, 4th generation ELISA is a cost effective method for blood donor screening.

KEYWORDS :

INTRODUCTION

Blood Transfusion is an essential part of clinical medicine, however each unit transfused carries the risk of transfusion-transmissible infections (TTIs) like Hepatitis B and C, HIV, Syphilis and Malaria and infrequently toxoplasmosis, brucellosis, viral infections like Cytomegalovirus, Epstein Barr Virus and Herpes.(1) All these diseases are known to cause significant mortality and morbidity along with a financial burden for both the affected person and the country.

The incidence of HIV, HBs Ag and HCV in blood donors varies in different states of the country and ranges from 0.1-0.9%, 0.86-2% and 0.28-0.53% respectively (2). HIV is the most grave TTI among transfusion transmitted infections mainly due to high viral content in one unit of donor blood, high rate of transmission of disease after transfusion of a HIV positive unit, the associated social stigma and lack of curative therapy.

It is very important to remain vigilant about the possible spread of these diseases through blood transfusion, keeping in mind their grave consequences. National AIDS Control Organization (NACO) estimates 86% HIV transmission is due to sexual route, 2.4% due to intravenous drug use, 2.0% due to blood and blood products, and 3.6% due to perinatal transmission in India. (3)

Transfusion associated HIV/AIDS is defined as those who were infected by HIV virus following blood transfusion after 1977, but they should be excluded from other risk factors like unprotected sexual contacts, needle prick injury, sharing of needles during tattooing and intra venous drug use and infection during pregnancy, at time of delivery and during breast feeding. Blood and blood product screening plays a vital role in preventing TTIs. In mid-nineties, percentage of HIV infection due to blood transfusion was as high as 8%. With advancement of screening methods worldwide, this percentage reduced to 1% in 2009. Indian blood donors showed a decrease in seroprevelance from 1.32% in 2000 to 0.3%in 2009.

In a developing country like India, a greater fraction of those infected may be unaware of their HIV positive status. These unidentified asymptomatic individuals unknowingly transmit the disease to others. Identification of this group either by donor questionnaire or blood screening is important to prevent transfusion transmitted HIV infection. According to drug and cosmetic act of India, donor screening for HIV infection is mandatory , since 1989.(4) An ideal screening test should be highly sensitive, be easy to perform, not require sophisticated instruments, be cost-effective and be able to distinguish between HIV-1 and HIV-2infections.

Fourth-generation assays permit the simultaneous detection of HIV antigen and antibody. With this test, it is possible to detect HIV p24 antigen during the window period, if the blood donor happens to be tested during the short peak of high levels of circulating virus particles. Although in theory the HIV antigen test can shorten the window period further, its use is also of limited value since there still remains a window of two weeks.

In our study, we compared 4th generation ELISA with a 4th generation Rapid immunochromatographic test (TRI-DOT) keeping HIV confirmation by RT-PCR as the gold standard. These assays are able to detect HIV-1 and HIV-2 antibodies as well as the p24 antigen, and can differentiate between HIV-1 and HIV-2 infections. We wanted to assess the potential use of 4th generation TRI-DOT as an alternative screening method during emergency situations by comparing with 4th generation ELISA which is one of the methods approved by NACO for blood donor screening.

Further, very limited literature is available both internationally & nationally, comparing 4th generation ELISA and the relatively recently introduced 4th generation rapid TRI-DOT + Ag test. Therefore, our study will go a long way in generating important data regarding the subject.

MATERIALS AND METHODS

In this prospective study, we screened a total of 1599 blood donors which included both whole blood donors and apheresis donors. All samples were screened with 4th generation ELISA (RFCL Limited), 4th generation Rapid Immunochromatograpy test (TRI-DOT+Ag, J. Mitra. & Co. Pvt. Ltd) and real time PCR technique. The sample size was calculated with 95% confidence interval estimation, 5% anticipated prevalence and 5% absolute error of margin.

No additional sampling was done, apart from that which was routinely collected in 5 ml sterile test tubes at the end of phlebotomy, for pre-transfusion testing. Ethical clearance was taken before conducting study from institutional ethical committee. The tests were performed on donor serum samples. Specimens with observable particulate matter were centrifuged prior to testing as suspended fibrin particles or

aggregates may yield false reactive results. According to our blood bank SOP and NACO guidelines all the samples were kept 4^0C till the time of testing, and all the sample were screened within six hours of collection.

RESULTS

The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/ or HIV-2 was determined by comparing the absorbance measured for each sample to the calculated cut-off value. Samples with absorbance values less than the cut-off values were considered to be ELISA nonreactive. Samples with absorbance values equal / greater than the cut-off value were considered to be ELISA reactive. Sample with absorbance values within 10% of cut off value were considered in grey zone. Samples initially found in grey zone were retested using the same ELISA kit with different batch number, and if again found in the grey zone were termed as possibly reactive and included as positive. If samples are found below the 10% of the cut off value it was considered as nonreactive during analysis.

INVALID TEST

If no DOT appears after the test is complete, either with clear background or with complete Bluish/yellowish background the test indicates error. This may indicate a procedural error or deterioration of specimen/reagents or particulate matter in the specimen. In our study, we screened a total of 1599 donors. Out of this 1438 (89.93%) were whole blood donors and 161 (10.07%) donors were SDP donors. Out of 1599 total donations 1578 (98.69%) were male donors and 21 (1.31%) were female donors. [Table 1]

Table 1: Sample Distribution vs. Gender

Sample Type		Gender		Total
		F	M	
Blood donors	Numbers	21	1417	1438
	% within Sample	1.5%	98.5%	100.0%
Platelet donors	Numbers	0	161	161
	% within Sample	0.0%	100.0%	100.0%
Total	Numbers	21	1578	1599
	% within Sample	1.3%	98.7%	100.0%
	% of Total	1.3%	98.7%	100.0%

Table-2: Summary of the Results

Sr No	Bag No	Absorbance Value 4^{TH} Gen ELISA	Result 4^{TH} Gen ELISA	Cut off Value	Clinical Hint 4^{TH} Gen ELISA	4^{TH} Gen TRI-DOT Result	HIV PCR
1	377	0.214	1	0.215	**Shadiness**	Negative	Positive
2	520	0.315	1.417	0.222	**Positive**	Negative	Positive
3	557	0.499	2.33	0.214	**Positive**	Negative	Positive
4	702	0.208	0.95	0.219	**Shadiness**	Negative	Positive
5	730	0.111	0.52	0.213	**Negative With Colour Change**	Negative	Negative
6	798	0.098	0.445	0.22	**Negative With Colour Change**	Negative	Negative
7	816	0.092	0.417	0.22	**Negative With Colour Change**	Negative	Negative
8	930	0.164	0.732	0.224	**Negative With Colour Change**	Negative	Negative
9	1094	0.108	0.498	0.218	**Negative With Colour Change**	Negative	Negative
10	1144	0.134	0.621	0.215	**Negative With Colour Change**	Negative	Negative
11	1254	0.121	0.559	0.217	**Negative With Colour Change**	Negative	Negative
12	P-SDP-64	0.08	0.352	0.227	**Negative With Colour Change**	Negative	Negative

In 4^{th} generation ELISA absorbance values of Bag numbers 520 and 557 were well above the cut off value and are considered as positive. Absorbance values of bag numbers 377 and 702 showed as shadiness initially and when repeated twice showed absorbance values well above the cut-off value and considered as positive. Bag number 730,798,816,930,1094,1144,1254 and P-SDP64 showed colour change but two repeat tests showed negative results well below the cut-off value and these were considered as negative during the study.

All samples positive by ELISA were also positive by PCR. No additional samples which were negative by ELISA were detected as positive by PCR. Chi-Square test could not be used to compare the distribution of results of the two devices (4^{th} Gen ELISA and 4th Gen TRI DOT), as the latter device gave all the results negative and failed to detect the positive cases. Therefore, nothing can be said with certainty about the effectiveness or sensitivity of the two devices.

Comparison of Proportions

Difference	95% CI	Chi-squared	DF	Significance level
0.26%	-0.0366% to 0.653%	2.401	1	P = 0.1213

Proportion of negative results given by 4^{th} Gen ELISA and 4th Gen TRI DOT were statistically compared (as there were no positives diagnosed by the latter method). The difference came out to be 0.26% which was found to be statistically not significant at p=0.1213 level of confidence. An ideal screening method should be highly sensitive and should be able to detect all the positive cases among those who have the disease. However here four cases detected by 4^{th} generation ELISA were not detected by the 4^{th} generation TRI-DOT.

Table 3: Sensitivity and Specificity-HIV Rapid TRI-DOT + Ag

	Disease Present	Disease Absent
Test Positive	0	0
Test Negative	4	1595
Total	4	1595
		(95% confidence interval)
Sensitivity	0.00%	0.00% to 60.24%
Specificity	100.00%	99.77% to 100.00%
Positive Likelihood Ratio	-	-
Negative Likelihood Ratio	1	1.00 to 1.00
Disease prevalence	-	-
Positive Predictive Value	-	-
Negative Predictive Value	99.75%	99.36% to 99.93%

There is no true positive detected by the 4^{th} generation TRI-DOT. Due to this, we could not calculate the sensitivity, Positive Likelihood Ratio, Positive Predictive Value and prevalence values for Rapid TRI-DOT. The statistical results of comparison of proportion were also not significant. Hence clinical significance cannot be ascertained by the present study with the given sample. The negative predictive value of rapid test is 99.75% (99.36% to 99.93%). This means that by using

TRI-DOT test we can find out 99.75% of those who do not have disease correctly. The two methods or devices are equally good to detect negative cases however the 4th generation TRI-DOT is still unsuitable for the detection of positive cases as a potential positive case may go unnoticed leading to more hazards than benefits.

Table 4: Sensitivity and Specificity-HIV 4th generation ELISA

	Disease Present	Disease Absent
Test Positive	4	0
Test Negative	0	1595
Total	4	1595
		(95% confidence interval)
Sensitivity	100.00%	39.76% to 100%
Specificity	100.00%	99.77% to 100.00%
Disease prevalence	0.25%	0.07% to 0.64%
Positive Predictive Value	100%	39.76% to 100%
Negative Predictive Value	100%	99.77% to 100.00%

The results of 4th generation ELISA when compared with the gold standard were corroborative. Hence there were no false positives or false negatives. Hence 4th generation ELISA is an ideal test for blood bank screening purpose, especially in a limited resource country like India where the cost of running PCR for all samples may be prohibitive.

DISCUSSION

HIV prevalence among Indian blood donor population is 0.084-3.87 per cent and according to NACO this is less as compared to general population. According to the various studies conducted in the country, HIV prevalence is less in voluntary donors as compared to replacement donors. (5)According to our study the prevalence of HIV is 0.25% which is higher as compared to that found in our previous study of three years duration (2003-2005). Our previous study conducted on blood donors revealed sero positivity rates of 0.12 per cent in 2003, 0.17 per cent in 2004 and 0.10 in 2005, with an average sero positivity of 0.13 per cent over three years.

In this study, four positive samples were detected by 4th generation ELISA, out of which two samples showed marginal positivity. All four samples were negative by 4th generation Rapid TRI-DOT +Ag, but positive with Real Time Polymerase Chain Reaction (RT-PCR) thereby implying a sensitivity of 4th gen ELISA comparable to RT-PCR, whereas sensitivity of 4th gen Rapid TRI-DOT was greatly inferior to both 4th gen ELISA and RT-PCR because of the inherent inability of the 4th gen RAPID to pick up cases of low antigenemia and low antibody titer as elaborated subsequently. The 4th gen Rapid test was unable to detect samples with E ratio of < 1:4.718, which can be detected by 4th gen ELISA (detect E ratio up to 1:1.506) and RT-PCR. 4th gen ELISA was able to TRI-DOT is able to give results within 10 minutes and may be useful for patients with high titer antibodies.

In our blood bank screening lab we used the NACO guideline as a reference material for preparing internal quality control for daily run. While preparing internal QC sample with borderline positivity (sample that has having E ratio 1.5-2.0) it has been seen that 4th generation TRI-DOT was able to detect upto1:4096 dilution (E ratio=4.718) and 4th generation ELISA was able to detect up to 1:16.384 dilution (E ratio=1.506).Therefore, 4th generation ELISA is more sensitive compared with the 4th generation TRI-DOT. However 4th generation ELISA is not able to find N subgroup of HIV-1 among our donors.

TRI-DOT can visually differentiate HIV-1 from HIV-2 infection and also detect p24 antigen. Appearance of HIV-1 and HIV-2 antibodies on the test device cannot rule out coinfections. Cross reactivity as high as 30% to 70 % due to sharing of common morphological and biological characteristics between HIV-1 and HIV-2 antibodies has been reported. Rapid TRI-DOT only requires reagent mixing with provided pipettes and does not require electric supply or other sophisticated instruments. Therefore this can be used to detect HIV infection in remote places and emergency situations. But because of low sensitivity this kit may not be able to detect positive cases with low viral titres. (E ratio less than 4). 4th generation ELISA is more sensitive and specific and more suitable for early detection of HIV in blood banking screening procedure. This is further corroborated by the fact that all four samples which were positive with 4th generation ELISA, also tested positive with gold standard RT-PCR.

TRI-DOT test showed strong positive result up to 1:4096 serum dilution during internal QC validation, therefore this test may be useful as an additional diagnostic tool in established cases of HIV/AIDS before starting anti retro viral therapy (ART) in voluntary counseling centers, as additional test for diagnosis of HIV in asymptomatic individuals [Strategy- III (algorithm-III) NACO guidelines], as second test for statistical purpose (strategy IIA NACO guidelines). A similar study conducted by T. Sudha at el. evidently confirmed the 4th generation ELISA as a sensitive tool and they found similar results as our study. During this study, the rapid test, was not able to detect six positive cases out of 1145 positive cases which were detected by 4thgen ELISA. All these six samples were p24 antigen positive however the rapid test used was for antibody detection only.

Nucleic acid technology can detect HIV RNA in pre seroconversion stage and significantly reduce the transmission of HIV via transfusion. NAT is considered as the gold standard for HIV screening among donors. However, use of universal NAT in a developing country like ours has limitations which include the infrastructure cost, highly trained technicians, higher cost per test, availability and time required to run the test. Presently the use of 4th generation ELISA is satisfactory as screening test for donors because of the above mentioned limitations in implementation of NAT, which also is not able to completely eliminate the transmission of HIV infection risk. Once P24 antigen appears in the blood it can be measured only for one and half month period after which it disappears.(6) TRI-DOT can be utilized to identify this stage of HIV infection which helps to initiate early treatment

The 4th generation ELISA has a lesser widow period due to its ability to detect antigens earlier than antibodies. When we use 4th generation ELISA alone there is a rare chance to miss the diagnosis due to second diagnostic window. This is extremely rare as it may occur due to delay in producing HIV specific antibodies once the P24 antigen disappears in the blood. When we use 4th generation ELISA during this situation there is a possibility to miss the HIV infected donor.

STRENGTH OF THE STUDY

In our study we prepared in house quality control samples and independently found the marginally positive titre for 4th generation ELISA and 4th generation rapid TRI-DOT by serial double dilutions technique. We conducted extensive quality control and ran these quality control samples with every test procedure. We observed that TRI-DOT test showed strong positive result up to 1:4096 serum dilution while 4th generation ELISA showed positive result till 1:16384 serum dilution. This showed that TRI-DOT + Ag test is inherently inferior to 4th generation ELISA.

The main limitation of our study is a relatively small sample size. Further studies with larger sample size may be taken up in future to arrive at a more definitive conclusion.

CONCLUSION

There have been continuing efforts to improve blood safety. Newer, faster and more sensitive tests have been developed in this quest. 4th generation TRI-DOT for HIV is one of such tests. During our study we found that it is greatly inferior to 4th generation ELISA. Four positive samples detected by 4th generation ELISA could not be detected by the 4th generation TRI-DOT. This suggest that 4th generation TRI-DOT is not suitable for blood banking as a screening method due to its low sensitivity.

All the four positive samples found by 4th gen ELISA were also confirmed by RT-PCR further augment our conclusion, that 4th generation ELISA should be continued for blood donor screening and rapid TRI-DOT should not be used as an alternative to 4th generation ELISA.

For a resource poor developing country like India, till the country is able to implement NAT/PCR, 4th generation ELISA is a cost effective method for blood donor screening.

In our study we did not find any HIV-2 infections. Several researches have shown that the sensitivity of 4th generation ELISA is reduced for detection of HIV-2 infection when it is combined with HIV-1 reagents. If we separately screen for HIV-2 alone with a separate kit then it is more sensitive for detection of HIV-2 infections. We conclude that this 4th generation ELISA kit does not provide desirable sensitivity for detecting the HIV-2 infection among blood donors.

Every technique has advantages and disadvantages therefore selecting best method for donor screening is a challenge. No single method is 100% effective. Judicial use of blood and blood component should always be encouraged.

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