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Comparative evaluation of ELISA & CLIA screening assays in the effective detection of HIV infection in blood donor samples: An observational study from a blood bank in tertiary health center

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***Abstract*---**Background: Since the set-up of the first blood bank in India in 1939, by the Bengal Red Cross Society, screening for transfusion transmissible infections in donated blood has been improvising steadily in the country. Currently it is either done by rapid diagnostic tests (RDTs) or the more common enzyme linked immunosorbent assay (ELISA) or the latest available sensitive assay namely, chemiluminescence immunoassay (CLIA). Aim: The prime objective of this analytical study was to assess the degree of performance of the readily available CLIA against two different ELISA testing methods for the serological screening of HIV. Methods: We have included 850 samples obtained from serial blood donors who donated blood dating from March 2021 to March 2022. All the collected blood samples were screened by two different ELISA testing methods & CLIA analyzer. The results were then computed and evaluated. Results: Out of 850 samples, 98 were ultimately confirmed to be HIV positive by qPCR testing. As far as sensitivity is considered, both CLIA and ELISA methods hadn't shown much disparity. But CLIA showed a higher specificity rate (CLIA: 99.6%, 749/752), concordance rate (CLIA:99.2%, 843/850), and positive predictive value (PPV) (CLIA: 94.4%, 92/98) than both the of ELISA assay kits we used in the study ($P < 0.05$).CLIA's kappa value was the highest among all the serologic assays (0.943). Conclusion: After conducting a comparative analysis, it is noted that CLIA assay is more specific in its accuracy for detecting HIV infection (antigen/antibody). This will

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include all the non-specifically reactive cases that were excluded by ELISA testing and thereby increase the donor sample count. Thus, CLIA can serve as a better screening method, more so in emergency conditions.

***Keywords*---**Specificity, accuracy, screening assay, sensitivity, HIV infection, ELISA, CLIA.

Introduction

One of the most important responsibilities of the blood transfusion services is to supply safe blood for transfusion. Thus in a country like India where HIV infection is much prevalent, it is compulsory to screen the donated blood for human immunodeficiency virus (HIV) antigens and antibodies, along with other transmissible infections like hepatitis B and C, syphilis and malaria, as recommended by the Drugs and Cosmetics Act (1940) The post transfusion risk of developing HIV is 5%–10% in an unscreened blood [1-3]. As the death toll due to HIV is still on the rise over the last decade, multiple screening modalities have been made available including Enzyme-linked Immunosorbent Assay (ELISA), Rapid Diagnostic Tests (RDTs), and Chemiluminescence Immunoassay (CLIA) and Electrochemiluminescence assay (ECLIA). Despite the multiple screening modalities available, each organization uses the most reliable assay to employ for screening of TTI [4,5]. While ELISA continues to be the most commonly used screening assay in India, newer methods like CLIA, being an automated innovation, is put in comparison for their performance and reliability [6-9].

Aim and objective

Taking into consideration the importance of screening assay in blood transfusion, we begin to evaluate the accuracy of the commercially available CLIA against two different ELISA kits (Gold standard) method in detecting HIV infection. Currently ELISA (for detecting antibodies), Rapid Diagnostic Tests RDTs (for detecting p24 antigens) and Nucleic Acid Test NAT (for detecting RNA) are usually utilized for screening HIV infection. The current study includes the latest advent in the country, namely the CLIA, which is an automated version of screening using either recombinant HIV antigens or antibodies. Our objective here is to compare the gold standard ELISA with the automated CLIA in effectively screening HIV infection in blood donor samples.

Materials and Methods

Our study design is a retrospective cross-sectional study and it was dating from March 2021 to February 2022 in the Transfusion medicine department at a teaching medical hospital. A count of 850 blood donations in the given study period were documented and the samples were each collected in a EDTA coated vial. The vials were then centrifuged and the separated plasma is retrieved in an aliquot of 3 ml to be screened by ELISA kits for detecting HIV (initial screening). Those samples which are non-reactive after the serological screening were released into the inventory as separate blood components. The reactive blood

samples were followed through a definite confirmatory algorithm (Fig.1), after which the confirmed HIV cases were run through two ELISA kits and CLIA for performance comparison.

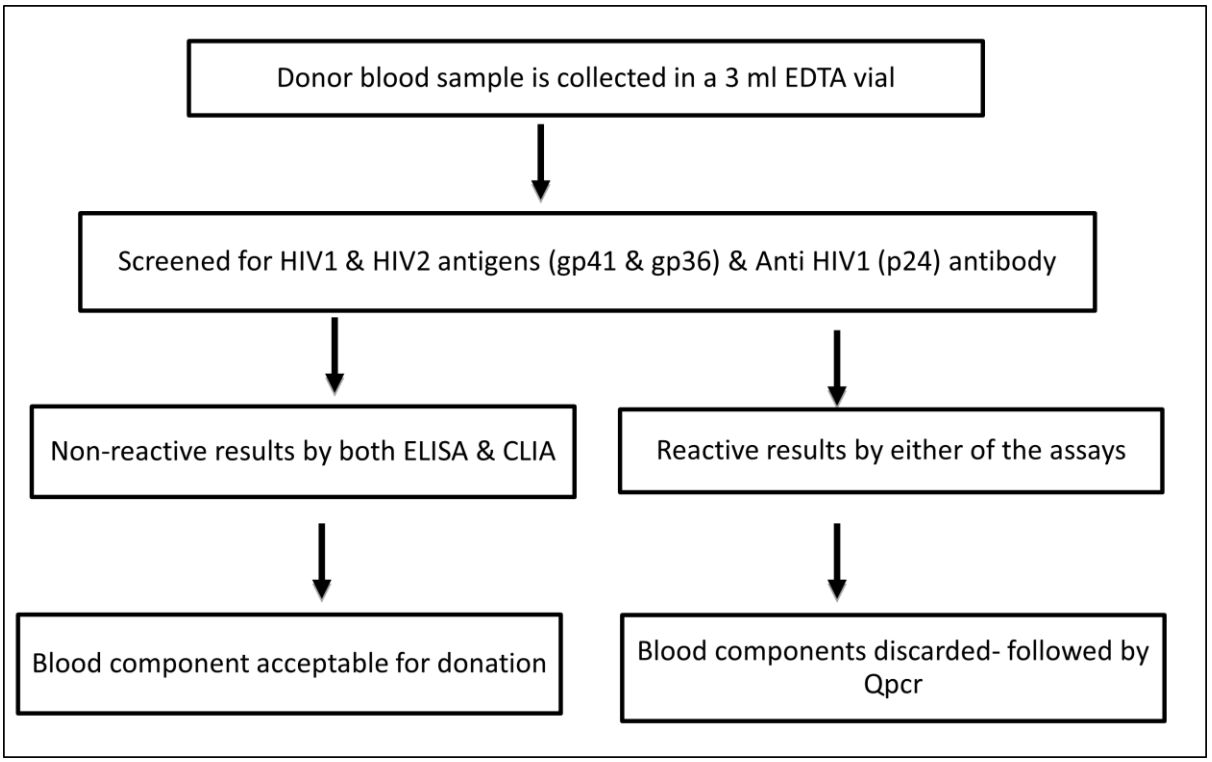

Figure 1: Study algorithm for detecting HIV infection

Table 1: Details of the serological screening assays employed in HIV detection

Type of assay	Branding of the kit	Specification of the assay	Markers detected
ELISA 1	BENE SPHERA	4th generation ELISA test	Anti HIV-1, anti HIV-2, p24 antigen (HIV-1)
ELISA 2	MICROLISA	4th generation ELISA test	Anti HIV-1, anti HIV-2, p24 antigen (HIV-1)
CLIA	Electra FA	Fully automated CLIA analyzer	Anti HIV-1, anti HIV-2, p24 antigen (HIV-1)

Statistical analysis

We used the IBM SPSS software (version 22 SPSS) for statistical calculations. Parameters including sensitivity, specificity, negative predictive value and positive predictive value were all calculated using standard formulae from the data collected. All values were clearly updated in an Excel spreadsheet for easier workflow. A statistically significant P value of less than 0.05 was adopted.

Results

A total of 850 whole blood donors (89% males & 11% females) were included in this study. The mean age was around 30 years (18–50 years). All 850 samples were run through the CLIA, two ELISA screening methods, in the same order. 97 donor samples were found reactive for HIV by CLIA method. 102 samples and 104 samples were found reactive for HIV in two ELISA testing methods respectively. None of these samples were found reactive for HbsAg, anti-HCV, malaria, or syphilis. The reactive samples (104) were sent for qPCr testing and thus 98 samples were confirmed as HIV positive cases As far as sensitivity is considered, both CLIA and ELISA methods hadn't shown much disparity. But CLIA showed a higher specificity rate (CLIA: 99.1%, 745/752), concordance rate (CLIA:99.2%, 843/850),andpositive predictive value (PPV) (CLIA: 94.4%, 92/98) than both the of ELISA assay kits we used in the study ($P < 0.05$).CLIA's kappa value was the highest among all the serologic assays (0.943). Tables 2 & 3 shows the comparative analysis between CLIA and ELISA in HIV screening

Table 2: Serological status of HIV1 & 2 antibodies and p24 antigen positive samples (n=790) tested by two ELISA assays against CLIA assay

Type of assay	Total no of reactive samples	Total number of non-reactive samples	Total number of samples confirmed as HIV
ELISA 1 & 2	108	742	98
CLIA	101	749	98

Table 3: Accuracy evaluation of Both ELISA assays against CLIA assay

Assay type	Markers detected	Sensitivity (%)	Specificity (%)	PPV %	NPV %	Concordance %	Kappa value
CLIA	Ag-Ab	100%	99.6%	94.4%	99.5%	99.2%	0.967
ELISA	Ag-Ab	99.2%	97.7%	98.1%	97.9%	97.5%	0.903

Discussion

Through the years, ELISA has been considered the mainstay and globally approved serological assay for HBsAg, HCV and HIV. While ELISA is religiously followed for screening TTIs in many centers throughout India, the much later introduced method; CLIA has taken an upper hand for its rapidity in screening large samples at once and for its automatic workflow. The present study was taken up to analyze the efficiency of CLIA over ELISA in detecting HIV infection, apart from its previously mentioned advantages. Interestingly, CLIA has been proven to have much better specificity and sensitivity in detecting HIV infection, through many recent studies worldwide [10–16]. To name a few, countries like Italy [17], US [18], Australia [10] and Sweden [16] have adopted routine donor blood screening for TTI by CLIA since a long time.

The present study evaluated the efficacy of CLIA by comparing it to 2 ELISA kits (4th generation) for detecting the presence of anti-HIV 1 & 2 and p24 antigen (HIV 1). All the parameters in detecting HIV infection were screened by the three assays. The samples were then sent for qPCR study to confirm the reactive cases. Thus, an evaluation table was made to compare specificity, sensitivity, PPV, NPV, concordance rate and kappa value between the two different methods. The follow up with NAT (qPCR) was essential after the donor samples were initially screened to note the true prevalence. Many donor samples that showed reactivity by ELISA screening were indeed non-specific when followed up by NAT [19].

Previous studies show that majority of samples having such non-specific reactivity were discarded and the donors were deferred [20]. The demand for blood transfusion remains to be at a higher rate than the rate of blood donation [21]. CLIA assays could readily decrease 97.7% of nonspecific reactions shown by ELISA. Thus, it is a proving point that CLIA like ECLIA, can be replaced for ELISA in avoiding unnecessary discard of blood showing non-specific reactions for TTI [22].

The current study agrees highly with a similar study from Italy, which employed anti-HIV antibodies and p24 antigen screening between CLIA and ELISA [10]. Many other studies stating that CLIA having higher sensitivity, specificity, positive predictive value and concordance rate in detecting HIV infection also coincides with the current study [15,23,24]. Similarly, the rate of specificity, positive predictive value and concordance rate were higher in 4th generation ELISA in detecting HIV infection as compared to its 3rd generation counterpart [25,26]. We have employed 4th generation ELISA in our study in contrast to CLIA which provides even better accuracy in detecting HIV infection.

ELISA, which is considered to be an open system assay, has fewer chances of optimum results, owing to its higher nonspecific reactivity rates. This could also be attributable to the high level of operational requirement which can vary from person to person, as opposed to the accurate and consistent workflow with automated assays like CLIA [27, 28]. One of the major advantages of CLIA or even ECLIA over ELISA assays is that, there is a great range of variability between each of the ELISA kit supplies. This variability can occur in the same ELISA kit running the same blood sample when done at different laboratories by different personals. Such variabilities which are highly operator based and which may lead to result bias is very negligible in a fully automated assay like CLIA. CLIA employs a controlled software system which automatically performs accurate planning and disposal of reagents and the values are uploaded accordingly in tabulation without any human intervention. The software is universal and does not vary from laboratory to laboratory and is fixed by the manufacturer, ultimately giving lesser opportunity for result bias or errors.

It was evident from our study that the two ELISA assay kits employed also showed much variation in the results in terms of PPV, specificity, concordance as compared to CLIA. The sensitivity of ELISA is well established in screening for HIV infection worldwide, and it is also evident through our study, where only two samples were missed by ELISA-1 and three samples were missed by ELISA-2. But considering the accuracy of CLIA in assessing the specificity, concordance and

NPV, CLIA still stands taller and proves better than CLIA. It is always the individual organization's approach, where the feasibility of the laboratory set up to adopt a high maintenance assay such as CLIA is put to scrutiny. Organizations with the infrastructure and capacity to maintain such automated assays should replace ELISA for routine TTI screening and utilize ELISA as an adjunct for confirmation for reactive samples.

Conclusion

As discussed earlier and in accordance with the results of the current study, it is seen that CLIA with full automation has more specificity than ELISA in detecting HIV infection, employing both antigen/antibody screening. The non-specific reactions can be avoided by approving CLIA as the serological screening modality to keep a large amount of blood samples from being deferred unnecessarily. While the CLIA assay requires more maintenance and higher monetary back-up as compared to the simple ELISA assay, there can be a large number of samples that can be screened at a single time. This counts for a decent amount of feasibility and time saving venture for laboratories which can accommodate the CLIA instrument in their set up. Lack of comparison with multiple ELISA kits from different laboratory set ups serve to be the limitation of our study, but similar results have been proven in a study from China, which utilized multiple ELISA kits in comparison to CLIA [14].

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Nil.

Conflicts of interest
There are no conflicts of interest.

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