Comparative assessment of commercial ELISA kits for detection of HIV in India

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Abstract

Background: India harbors the 3rd highest HIV infected population globally. The magnitude of the HIV detection challenge is enormous. ELISA is the most commonly used screening technique for HIV. There is always an acute need for good quality ELISA kits. However, the quality evaluation data on Indian kits are very limited in comparison with internationally recognized kits. This study aimed to evaluate the performance and diagnostic usefulness of five commercially available ELISA kits which are frequently used in India.

Findings: The ELISA kits evaluated using an in-house well characterized 100 member sera panel revealed 100% sensitivity for all the batches. However, batch to batch variation in terms of specificity, positive predictive value (PPV) and efficiency, although not statistically significant (p > 0.05), was observed. For specificity, the 3rd generation kits (mean 99.6% to 99.3%) were comparatively better than the 4th generation assays (97.2% to 96.9%). But the 4th generation kits performed far better in the ability for early detection post HIV infection in the 25 member commercial seroconversion panel with a margin of at least 22 days and as high as 35 days than the 3rd generation assays.

Conclusions: The commercial ELISA kits with 100% sensitivity seem appropriate for HIV screening. The ability of early detection post HIV infection favors use of 4th generation kits for ensuring HIV free blood for transfusion. Lot to lot variations, especially kits having the specificity level ≤ 98.0%, indicate the need for a regular mechanism of kit evaluation for each batch for procuring kits appropriate for intended use.

Keywords: HIV, ELISA, Sensitivity, Specificity, Efficiency, Sera panel, Seroconversion panel

Findings

Background

HIV is a major global public health issue [1]. For assuring a safe blood supply and preventing HIV infection, proper and accurate detection of HIV is essential [2]. In India, diagnosis of HIV infection is a major challenge [3,4]. Several commercial assays are available for detection of HIV infection. ELISA is the most commonly used screening assay for HIV [2,5]. A number of ELISA kits for HIV detection with different principles are available. Nowadays, in India 3rd generation ELISA are most commonly used. The 4th generation assays are based on combined detection of antigen and antibodies simultaneously and reduce the diagnostic window period further, compared to third generation ELISA which is based on anti-HIV antibody assay [6-8]. The improved sensitivity for ELISA is mostly accompanied by a decreased specificity.

In an Indian perspective, limited articles on evaluation and performance of ELISA kits are available [9] though HIV testing is being done for a vast numbers of individuals as well as large number of specimens for ensuring HIV free safe blood for transfusion. Being the 2nd most populous country with the 3rd largest burden of HIV in the world [10], the magnitude of HIV testing challenge in India is enormous and the appropriate response to the challenge is to ensure the quality of the assay kits suitable for the intended use. This study aims to evaluate the quality of commonly available commercial ELISA kits for their ability to detect HIV suitable for appropriate use in India.

Materials and methods

The study was carried out at a National HIV Reference Laboratory designated for evaluation of diagnostic kits, including ELISA, in India. A well characterized, 100 members, in-house HIV serum panel was used to evaluate and compare the performance of the kits. The sera used for
preparing the in-house panel were obtained anonymously from attendees of the Counseling and Testing Centre by taking informed consent as per the protocol approved by Institutional Ethical Committee of National Institute of Cholera and Enteric Diseases. Beside the negative and positive sera, this serum panel also contained low positive sera that have shown uniform results in all assays used for validation. The characterization of in-house panel was done using United States Food and Drug Administration (U.S. FDA) or Indian Central Drug Standard Control Organization (CDSCO) approved kits (Table 1). Samples non-reactive for all assays were defined as negative and reactive for all assays were defined as positive member in the panel. A commercial seroconversion panel (Lot# RP-018, Bio-Rad Laboratories, U.S.A) used to evaluate kits consists of a series of 25 specimens collected from an individual infected with HIV undergoing seroconversion.

| Source of sample | Name of panel | Test details | Confirmatory test |
|------------------|---------------|--------------|-------------------|
| ICTC | HIV Sera Panel (In-house) | Genetic Systems, Genedia HIV Ag-Ab ELISA, Rapid 1: Determine HIV1/2 | Recombinant Immunoblot Assay |
| | | HIV-1/HIV-2, Green Cross Life Science Corp. Korea, Inverness Medical Japan Co. Ltd. Japan | Chiron RIBA HIV-1/HIV-2 SIA |
| | | Bio-Rad Laboratories, USA, Reactivity range (pos specimens): 3.503-8.899 | Ortho Clinical Diagnostics Ltd, USA |
| | | | AMPLICOR HIV-1 DNA Test, Version 1.5 |
| | | J. Mitra & Co. Pvt. Ltd. India | Roche Molecular Systems Inc, USA |

Table 1: Details of kits used for characterization of in-house panel sera

| Source of sample | Name of panel | Test details | Confirmatory test |
|------------------|---------------|--------------|-------------------|
| ICTC | HIV Sera Panel (In-house) | Genetic Systems, Genedia HIV Ag-Ab ELISA, Rapid 1: Determine HIV1/2 | Recombinant Immunoblot Assay |
| | | HIV-1/HIV-2, Green Cross Life Science Corp. Korea, Inverness Medical Japan Co. Ltd. Japan | Chiron RIBA HIV-1/HIV-2 SIA |
| | | Bio-Rad Laboratories, USA, Reactivity range (pos specimens): 3.503-8.899 | Ortho Clinical Diagnostics Ltd, USA |
| | | J. Mitra & Co. Pvt. Ltd. India | AMPLICOR HIV-1 DNA Test, Version 1.5 |
| | | | Roche Molecular Systems Inc, USA |

Table 2: Performance of HIV ELISA kits with In-house Sera Panel

| Total no. of samples | Total no. of lots evaluated | Kit performance |
|----------------------|-----------------------------|-----------------|
| 100                  | 5 Lots                      | J. Mitra & Co. Pvt. Ltd. | Span Diagnostics Ltd. | Transasia Bio-medicals Ltd. | Bio-Rad Laboratories. | Biomerieux. |
| (Confirmed Positive = 40 Confirmed Negative = 60) | for each kit | Assay result | Assay result | Assay result | Assay result | Assay result |
| | | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg |
| Lot 1 | Pos | 40 | 00 | 40 | 01 | 40 | 00 | 40 | 02 | 40 | 02 |
| | Neg | 00 | 60 | 00 | 59 | 00 | 60 | 00 | 58 | 00 | 58 |
| Lot 2 | Pos | 40 | 01 | 40 | 00 | 40 | 00 | 40 | 02 | 40 | 02 |
| | Neg | 00 | 59 | 00 | 60 | 00 | 60 | 00 | 58 | 00 | 58 |
| Lot 3 | Pos | 40 | 00 | 40 | 00 | 40 | 00 | 40 | 01 | 40 | 01 |
| | Neg | 00 | 00 | 00 | 60 | 00 | 60 | 00 | 58 | 00 | 58 |
| Lot 4 | Pos | 40 | 01 | 40 | 00 | 40 | 00 | 40 | 01 | 40 | 03 |
| | Neg | 00 | 59 | 00 | 60 | 00 | 60 | 00 | 59 | 00 | 59 |
| Lot 5 | Pos | 40 | 00 | 40 | 01 | 40 | 01 | 40 | 02 | 40 | 01 |
| | Neg | 00 | 60 | 00 | 59 | 00 | 59 | 00 | 58 | 00 | 59 |

Five batches each of 5 commonly available commercial ELISA kits, including 3rd and 4th generations, for HIV detection were evaluated (Table 2). All the kits were tested and results were validated strictly adhering to manufacturers’ instruction. The evaluation process maintained an unbiased method following a double blind procedure by using different personnel for pre-analytical and analytical testing sections. The final analysis of results with interpretation was done by the laboratory in-charge. The status of samples was unknown to the persons involved in pre-analytical and analytical procedures. The performance of kits was evaluated and compared in terms of sensitivity ([TP/(TP + FN)]×100), specificity ([TN/(TN + FP)]×100), positive predictive value (PPV = [TP/(TP + FP)]×100), negative predictive value (NPV = [TN/(TN + FN)]×100) and efficiency ([TP + TN)/(TP + FN + TN + FP)]×100), where TP = number of true positives, TN = number of true negatives, FP = number of false positives and FN = number of false negatives.
of false negatives [11]. Confidence Interval (CI) was used to address precision of the proportion estimates and the degree of confidence was set to 95% [12]. Chi-square analysis was performed to assess the variation for specificity, PPV and efficiency among different kits as well as in different batches of same kit.

Results
None of the ELISA kits evaluated was able to identify all the panel members correctly by all the batches (Table 2). But all the kits were found to be 100% sensitive in all the batches. Variation in batches of all the kits was evident in terms of specificity, PPV and efficiency (Table 3). ERBA LISA HIV 1 + 2 provided correct results in 4 batches by identifying all panel samples correctly with 99.8% efficiency. Microlisa HIV and Enzaids HIV 1 + 2 both performed equally by correct result only in 3 batches with 99.6% efficiency. The 4th generation kits, Genscreen and Vironostika, showed the false positivity rates higher than the 3rd generations, but the variations were not statistically significant in terms of specificity ($\chi^2 = 0.0683$, df = 4, p > 0.05), PPV ($\chi^2 = 0.1253$, df = 4, p > 0.05) and efficiency ($\chi^2 = 0.0230$, df = 4, p > 0.05) of all batches of all the ELISA kits. The performance of the kits evaluated using seroconversion panel revealed that all the 3rd generation kits showed equal sensitivity by detecting HIV positivity. In contrast, the 4th generation kits, Genscreen (detected panel member 5, day 16) and Vironostika (detected member 7, day 29) were significantly more sensitive and were able to detect HIV positivity 35 and 22 days earlier respectively than the 3rd generation ELISA kits (Table 4).

Discussion
ELISA is the type of test most commonly used for detection of HIV particularly for large numbers of specimens

Table 3 Performance characteristic of HIV ELISA kits used for comparative evaluation

| Kit Name & Company | Lot | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Efficiency (%) |
|-------------------|-----|----------------|----------------|---------|---------|----------------|
| Microlisa HIV Company: J. Mitra & Co. Pvt. Ltd. | 1 | 100 | 99.3 | 100 | 99.0 | 100 | 99.6 |
| | 2 | 100 | 98.3 | 97.6 | 100 | 99.5 | 100 | 99.6 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 4 | 100 | 98.3 | 97.6 | 100 | 100 | 100 |
| | 5 | 100 | 100 | 100 | 100 | 100 |
| Enzaids HIV 1 + 2 ELISA Company: SPAN Diagnostics Ltd. | 1 | 100 | 98.3 | 97.6 | 100 | 99.5 | 100 | 99.6 |
| | 2 | 100 | 98.3 | 97.6 | 100 | 100 | 100 | 99.0 |
| | 3 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 4 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 5 | 100 | 98.3 | 97.6 | 100 | 100 | 100 |
| ERBA LISA HIV 1 + 2 Company: Transasia Bio-medicals Ltd. | 1 | 100 | 96.6 | 95.4 | 100 | 98.0 | 100 | 98.4 |
| | 2 | 100 | 96.6 | 95.4 | 100 | 100 | 100 | 98.0 | 97.7-99.0 |
| | 3 | 100 | 98.3 | 97.6 | 100 | 99.0 |
| | 4 | 100 | 98.3 | 97.6 | 100 | 99.0 |
| | 5 | 100 | 96.6 | 95.4 | 100 | 98.0 |
| Genscreen Plus HIV Ag-Ab ELISA Company: Bio-Rad Laboratories, U.S.A | 1 | 100 | 96.6 | 95.4 | 100 | 98.0 | 100 | 98.2 |
| | 2 | 100 | 96.6 | 95.4 | 100 | 100 | 100 | 98.0 | 97.2-99.1 |
| | 3 | 100 | 98.3 | 97.6 | 100 | 99.0 |
| | 4 | 100 | 95.0 | 93.4 | 100 | 97.0 |
| | 5 | 100 | 98.3 | 97.6 | 100 | 99.0 |

CI: Confidence Interval.
[2]. But the discordance between the results of different ELISA kits as well as in different lots of the same kit (particularly false positive rates), as evident in this study, highlights an important problem of potentially causing stress to falsely-positive individuals and may also lead to additional expenses [13,14]. Hence, evaluation of diagnostic ELISA kits gains importance for ensuring the availability of suitable kits with better performance in terms of recommended sensitivity and specificity [11], as in case of blood bank testing where a high degree of sensitivity is also recommended for choosing the testing kit [11]. Better performance, comparatively, was observed for ERBALISA kits with 100% efficiency in 4 out of 5 batches (mean efficiency 99.8%). Microlisa and Enzaids each showed mean efficiency of 99.6%. The performance of Genscreen and Vironostika was compromised in terms of specificity, PPV and efficiency as these kits give few false positive results. The PPV as estimated based on the composition of panel sera will change according to the prevalence in the targeted population to be tested [9]. Thus, in an Indian scenario with 0.27% HIV prevalence [15], the estimated PPV of the HIV ELISA kits would be 40.30% for both J.Mitra & Co. Pvt. Ltd. and Span Diagnostics Ltd., 57.40% for Transasia Bio-Medicals Ltd. and 14.40% for Bio-Rad Laboratories. The performance of all five kits in terms of NPV favors their use as a primary screening assay for HIV infection. Unique combination of simultaneous Ag-Ab assay gives better performance in a seroconversion panel as well as reducing the testing window period by 3.82 days on average [6]. In this study, Genscreen and Vironostika, both 4th generation assays, outperformed the 3rd generation ELISA by reducing the window by 35 and 22 days respectively in the seroconversion panel. Genscreen showed better performance and identified early seroconversion than Vironostika in another study [16]. The 3rd generation kits demonstrated

| Panel member Bleed day | J Mitra & Co. Pvt. Ltd. | Span Diag. Ltd. | Transasia Bio-Medicals Ltd. | Bio-Rad | Biomerieux |
|------------------------|------------------------|----------------|-----------------------------|---------|----------|
| MICROLISA HIV (S/Co)   | ENZAIDS HIV 1 + 2 (S/Co) | ERBALISA (S/Co) | GENSCREEN (S/Co) | VIRONOSTICA (S/Co) |
| 1                      | 0.022                  | 0.235          | 0.258                      | 0.224   | 0.238    |
| 2                      | 0.007                  | 0.162          | 0.183                      | 0.221   | 0.15     |
| 3                      | 0.007                  | 0.177          | 0.19                       | 0.238   | 0.112    |
| 4                      | 0.015                  | 0.148          | 0.171                      | 0.238   | 0.254    |
| 5                      | 0.015                  | 0.162          | 0.167                      | 2.425   | 0.233    |
| 6                      | 0.007                  | 0.162          | 0.171                      | 2.888   | 0.258    |
| 7                      | 0.065                  | 0.877          | 0.86                       | 3.291   | 2.002    |
| 8                      | 6.723                  | 4.401          | 3.806                      | 9.396   | 2.601    |
| 9                      | 7.511                  | 2.523          | 2.665                      | 12.724  | 2.436    |
| 10                     | 7.285                  | 3.65           | 4.182                      | 14.224  | 2.967    |
| 11                     | 5.226                  | 5.549          | 4.601                      | 16.388  | 2.95     |
| 12                     | 7.635                  | 5.069          | 5.247                      | 17.993  | 3.245    |
| 13                     | 6.211                  | 5.339          | 5.277                      | 19.828  | 3.026    |
| 14                     | 6.073                  | 6.419          | 5.787                      | 20.91   | 4.513    |
| 15                     | 6.825                  | 3.552          | 3.76                       | 21.045  | 4.189    |
| 16                     | 7.182                  | 4.639          | 4.95                       | 22.425  | 4.472    |
| 17                     | 7.328                  | 4.177          | 4.563                      | 22.993  | 5.386    |
| 18                     | 7.81                   | 3.953          | 4.19                       | 22.037  | 5.729    |
| 19                     | 5.401                  | 3.639          | 4.224                      | 23.187  | 4.72     |
| 20                     | 7.606                  | 3.964          | 4.285                      | 23.575  | 5.604    |
| 21                     | 7.372                  | 5.141          | 5.635                      | 23.56   | 5.976    |
| 22                     | 6.467                  | 3.469          | 3.798                      | 24.007  | 5.988    |
| 23                     | 7.562                  | 4.372          | 4.688                      | 29.881  | 6.254    |
| 24                     | 7.993                  | 3.776          | 4.213                      | 24.91   | 6.537    |
| 25                     | 8.825                  | 7.415          | 7.988                      | 24.858  | 6.625    |

S/Co = Sample/cut of.
efficiency ranging from 98.6 to 99.8%. Though the efficiency of the 4th generation assays is lower, the high sensitivity demonstrated by the kits may favor them for HIV screening purposes, because of their early detection by about 3 weeks post infection. Although lot to lot variation is evident in the study it is not statistically significant \((p > 0.05)\), but kits with specificity level <98% are not recommended for diagnosis of HIV infection in India according to the national guideline [11].

The panel size is small with 100 members and one seroconversion panel only. Kit evaluation with small panel size may be valuable where studies are limited [17]. Use of a range of seroconversion panels is essential to test the biological differences in the timing of appearance of different antibodies to specific antigens in the host response to the various HIV antigens in a range of individuals. A more robust assessment requires the testing of, for example, 10 different seroconversion panels [18,19].

Conclusion
With 100% sensitivity, both the 3rd and 4th generation commercial ELISA kits seem are appropriate as screening assay for detection of HIV infection. Earlier detection post HIV infection favors the use of 4th generation kits for ensuring HIV free blood for transfusion. The lot to lot variation in terms of specificity warrants batch pre-acceptance testing of all new lots or batches of commercially available ELISA kits in India to ensure that new batches perform as well as previous ones.

Abbreviations
AIDS: Acquired Immuno Deficiency Syndrome; CDSCO: Central Drug Standard Control Organization; ELISA: Enzyme Linked Immunosorbent Assay; HIV: Human Immunodeficiency Virus; NPV: Negative Predictive Value; PPV: Positive Predictive Value; USFDA: United States Food and Drug Administration.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
All the authors conceived the study, SN, SM and SCB carried out the laboratory work, MKS analyzed and interpreted the data, SM performed the statistical analysis and all the authors contributed in drafting, and critically reviewed and approved the final manuscript.

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