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Analytical and clinical performance evaluation of enhanced chemiluminescence-based fourth-generation HIV combo assay: Report from tertiary health-care setup in North India

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Abstract:

INTRODUCTION: HIV fourth-generation assay, designed for the detection of HIV p24 antigen along with anti-HIV antibodies of both immunoglobulin M and immunoglobulin G type against HIV 1 and HIV 2 viral antigens, have helped in the early detection of HIV infection and supports in minimizing the transmission risk in the acute phase of infection. The objective of this study was to evaluate the analytical and clinical performance of HIV fourth-generation assay based on enhanced chemiluminescence technology.

MATERIALS AND METHODS: The analytical performance of the assay was evaluated in terms of accuracy, precision, limit of detection, type of sample (serum vs. plasma), cross-reactivity (with other transfusion transmissible infections markers), and interference (with endogenous substances). Proficiency control material included kit-controls, archived known positive donor samples, third-party controls, and World Health Organization (WHO)/National Institute for Biological Standards and Controls (NIBSC, MHRA, UK) controls. The clinical performance was evaluated using routine donor and patient samples received during the study period.

RESULTS: HIV fourth-generation assay showed reliable and reproducible results measured in terms of coefficient of variation % with kit-controls, archived known positive donor samples, third-party controls, and WHO international standards for anti-HIV 1 and 2 antibodies, HIV1 p24 antigens and HIV2 p26 antigen controls. The analytical sensitivity of the HIV fourth-generation assay was found to be 0.1 IU/mL of HIV1 p24 antigen control and there was no cross-reactivity or interference observed. In the clinical performance of the assay, HIV fourth-generation assay showed reliable performance in both donor and patient samples.

CONCLUSION: HIV fourth-generation assay meets the requirements for its use as a screening assay for HIV infection based on the analytical and clinical performance of the assay.

Keywords:

Graves ophthalmopathy, graves orbitopathy, thyroid eye disease, rituximab, teprotumumab, tocilizumab

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Introduction

India has third-largest population of people with HIV infection in world, with 2.35 million people living with HIV, accounting for 0.22% of people in the 15–49 years age group, according to prevalence estimates released by National AIDS Control Organization (NACO), India in 2019.[1] Though new HIV infections have declined, the decline is only 27% between 2010 and 2017, which is way below the intended target of NACO in achieving a 75% reduction by 2020 from 2010 levels.[2]

Established modes of HIV transmission are sexual, parenteral (through blood transfusion, contaminated needle, or organ transplant), and vertical (mother-to-child) route. More common modes of transmission include heterosexual contact followed by parenteral and vertical.[3,4] Effective screening and detection of HIV infection in the early asymptomatic stage are important to prevent transmission of infection through any of these routes.

Blood is a very effective route for the transmission of HIV infection. Shaw and Hunter[4] reported that this route has a very high probability of transmission and its estimated contribution is 2.6 million cases worldwide. Screening protocol in blood and organ donors, in India, includes mandatory testing for anti-HIV 1 and 2, anti-HCV, HBsAg, serological tests for syphilis, and test for malaria.[5] Mandatory screening for these five transfusion transmissible infections (TTI) ensures the safety of blood components/organs for transfusion or transplant.

The technological advancement in the form of a newly introduced fourth-generation assay has been designed for the detection of both anti-HIV antibodies against HIV-1 and HIV-2 and HIV-1 p24 antigen. This fourth-generation assay has helped in narrowing down the diagnostic window period from 3–4 weeks to 2–3 weeks.[6] This significantly enhances early detection of HIV infection and helps in minimizing transmission risk during the early phase of infection.[7] Centers for Disease Control and Prevention (CDC) updated their HIV testing guidelines and recommended that laboratories should conduct initial testing for HIV with the Food and Drug Administration-approved fourth-generation HIV immunoassay for HIV-1 and-2 infection.[8]

The objective of this study was to evaluate the analytical and clinical performance of enhanced chemiluminiscence immunoassay (CLIA)-based fourth-generation HIV immunoassay.

Materials and Methods

Settings

The study was carried out in the department of Transfusion Medicine in a tertiary care hospital in the national capital region, India, between January 2018 and December 2020.

Materials

Chemiluminescence-based fourth-generation HIV immunoassay

This fourth-generation assay (VITROS HIV Combo, OCD, Raritan, NJ, US) is also referred to as "HIV Combo assay."[9] This assay uses three recombinant antigens derived from the HIV envelope (HIV-1 group M envelope [env 13], HIV-1 group O envelope [env 70–3] and HIV-2 envelope [env 31]) for simultaneous detection of antibodies to HIV-1 and HIV-2 subtype. In addition, mouse monoclonal anti-HIV p24 antibodies were also included in the immunoassay to enable the detection of HIV-1 p24 antigen.

Equipment

Calibrated automated CLIA-based analyzer (VITROS 3600, OCD, Raritan, NJ, US) was employed for testing performed in this study. Assay calibration was routinely performed with its lot-specific calibrator, following the manufacturer's instructions.

Proficiency control material

Five types of proficiency control material used in this analytical study were as follows:

1. Kit-controls (anti-HIV antibody controls, VITROS, OCD, Raritan, NJ, US) – Three different lots were used (lot number 790, 808, and 810). Each of these lots had three different controls; one negative antibody (anti-HIV-1 and -2) control and two positive antibody (anti-HIV-1 and -2) controls
2. Archived, known positive donor samples (anti-HIV-1 antibody) – Three different samples (samples 1, 2, and 3)
3. Third-party controls – One anti-HIV antibody positive (Virotrol, lot number 119090) and one HIV negative control (Viroclear, lot number 107690), and one HIV p24 antigen positive control (lot number 114870); Bio-Rad, Hercules, California, US)
4. World Health Organization (WHO)/National Institute for Biological Standards and Controls (NIBSC, MHRA, UK) controls – Negative control included one diluent control and positive controls included one anti-HIV-1 antibody first international reference control (NIBSC Code: 02/210), one anti-HIV-2 antibody control (NIBSC code: 99/674), one HIV-1 p24 antigen control (NIBSC Code: 90/636), and one HIV-2 p26 antigen control (NIBSC code: 16/236)

5. Donor and patient samples – Routine donor and patient samples (serum samples) received during the study period were analyzed for the evaluation of clinical performance.

Methods

Study design

The study comprised prospective analytical performance evaluation in terms of accuracy, precision, limit of detection (LOD), type of sample (serum vs. plasma), cross-reactivity (with other TTI markers), interference (with endogenous substances), and clinical performance.

Accuracy verification

Accuracy was verified by testing multiple controls for anti-HIV-1 and anti-HIV-2 antibodies and p24 antigen, including kit-controls, archived known positive samples, third-party controls, and NIBSC controls. Verification plan involved comparing "expected" and "actual" results. In addition, accuracy was also verified using controls from different lots (kit-control for antibodies [one negative and two positives] and third-party control for p24 antigen [one positive]). Kit controls from three different lots were run in five replicates on 5 consecutive days. For each type of control, intra-day, and inter-day, mean, standard deviation (SD), and coefficient of variation (CV) were calculated and analyzed.

Precision verification

Short-term and long-term precision verification of immunoassay was based on assessment criteria defined in National Committee for Clinical Laboratory Standards (NCCLS) document EP15-A3.[10] Kit-control for antibodies (one negative and two positives) and third-party control for p24 antigen (one positive) were used for the precision verification study. Like accuracy verification, kit controls from three different lots were run in five replicates on 5 consecutive days. For each type of control, intra-day, and inter-day, mean, SD and CV were calculated and analyzed.

Limit of detection

LOD was performed for HIV p24 antigen only. It was evaluated by using serial dilutions of HIV-1 p24 antigen (NIBSC control) in human AB group serum samples (negative for all TTI markers). The dilutions tested were having approximate concentrations of 100 IU/mL, 10 IU/mL, 1 IU/mL, 0.1 IU/mL, and 0.01 IU/ml. NIBSC claims an overall sensitivity of ≤0.48 IU/mL (lower LOD) for the HIV-1 p24 antigen standard (90/636).

Sample type

To identify the appropriate sample type for use, ten consecutive donor samples were collected in clotted tubes (serum; 4 ml serum tube, BD Vacutainer®, New Jersey, US) and anti-coagulated tube (plasma; EDTA, 3 ml BD Vacutainer®, New Jersey, US), respectively, were screened with the assay in parallel and results were compared in terms of sample to the cut-off ratio (S/Co). In addition, ten replicates of a single archived known positive sample (both serum and plasma aliquots) were also screened.

Cross-reactivity

Cross-reactivity study was carried out to verify any cross-reaction with other common TTI markers.

Table 1: Accuracy verification of HIV Combo assay using controls from single lot

Type of controls	Name of controls	Expected value (S/Co)	Value obtained (S/Co)	Expected result	Actual result
Kit control (lot 790)	Negative control	0.2 (0.0-0.4)	0.25	Nonreactive	Nonreactive
	Anti-HIV 1 positive control	12.3 (8.24-16.36)	12.55	Reactive	Reactive
	Anti-HIV 2 positive control	23.9 (16.02-31.78)	22	Reactive	Reactive
Archived known-positive samples	Sample 1	105	104.8	Reactive	Reactive
	Sample 2	107.8	108.0	Reactive	Reactive
	Sample 3	58.2	57.9	Reactive	Reactive
Third-party control	Negative control	0.3 (0.1-0.5)	0.31	Nonreactive	Nonreactive
	HIV p24 antigen control	3.02 (1.2-4.84)	4.44	Reactive	Reactive
	Anti-HIV 1 positive control	2.58 (1.2-3.96)	2.94	Reactive	Reactive
	Anti-HIV 2 positive control	2.6 (1.2-4.0)	2.44	Reactive	Reactive
WHO/NIBSC International Standard control	Diluent control	NA	0.31	Nonreactive	Nonreactive
	Anti-HIV-1 subtype A (Group M)	NA	65.5	Reactive	Reactive
	Anti-HIV-1 subtype B (Group M)	NA	113	Reactive	Reactive
	Anti-HIV-1 subtype C (Group M)	NA	76.35	Reactive	Reactive
	Anti-HIV-1 subtype E (Group M)	NA	49.85	Reactive	Reactive
	Anti-HIV-1 Group O	NA	6.27	Reactive	Reactive
	Anti-HIV-2	NA	65.59	Reactive	Reactive
	HIV 1 p24 antigen	NA	995	Reactive	Reactive

WHO=World Health Organization, NIBSC=National Institute for Biological Standards and Controls, S/Co=Sample to cut-off ratio, NA=Not available

Known TTI-positive samples, including HBsAg, anti-HCV antibody, anti-syphilis antibody, and anti- cytomegalovirus (CMV) antibody immunoglobulin G (IgG), ten samples each, were tested to exclude cross-reactivity. We also included ten samples from repeat donors with a history of COVID-19 infection (positive for anti-SARS CoV2 IgG antibody) to assess the cross-reactivity of anti-SARS CoV2 IgG antibody with HIV combo assay. In addition, the WHO first international reference reagent for HIV-2 p26 antigen (NIBSC code: 16/236) was also tested to verify any cross-reactivity with the p24 antigen. This reference material contained HIV-2 p26 virus-like particles derived from a clinical isolate.

Interference

Three donor samples, one each, having a high index of hemolysis (hemolysis index = 104), icterus (icteric index = 15), or lipemia (turbidity index = 20) as determined by automated CLIA analyzer were identified. In parallel, one-donor sample each, negative for HIV (negative for anti-HIV-1 and-2 antibodies and p24 antigen) and positive for HIV (positive for anti-HIV-1 and-2 antibodies) were also identified. In an experimental setting, these three samples with endogenous substance (hemolyzed RBC, bilirubin, lipid) were separately spiked with the identified negative and positive samples in a 1:1 ratio. These six samples were then re-analyzed by the assay to verify any interference due to endogenous substance, and results (S/Co) obtained in re-run were compared with the result (S/Co) from previous un-spiked samples.

Clinical performance

To verify the clinical performance of the HIV Combo assay, routinely received samples of both, apparently healthy blood donors and patients were screened in the assay as per the manufacturer's instructions. All "reactive" samples were considered "confirmed positive," when reactive by western blot (HIV 1 and 2 Western Blot, J Mitra and Co. Pvt. Ltd., New Delhi, India). The rate of false positivity was defined as the percentage of the difference between "confirmed positive" and "reactive" samples as the numerator and the total number of samples as the denominator.

Statistical methods

Data entered and analyzed in Microsoft Excel (MS Office 2016, Microsoft, USA) did not have any personal identifiers, and complete confidentiality was maintained. Anonymized patient and donor samples were used for testing. SPSS software (Version 26, IBM India Pvt. Ltd., Bengaluru, Karnataka, India) was used for statistical evaluations. Mean, range, percentage, SD, and CV% were calculated for continuous data.

Table 2: Accuracy verification of HIV Combo assay by using controls from different lots

Reagent lot	Kit-controls						Third party controls	
	HIV negative kit-control		HIV-1 antibody positive control		HIV-2 antibody positive control		Virotrol HIV p24 antigen control	
	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual
Lot 1	0.2 (0-0.4)	0.26 (0.2-0.33)	10.3 (6.9-13.7)	12.55 (11.75-13.47)	20.1 (13.46-26.74)	22.0 (2.83-22.95)	3.02 (1.2-4.84)	4.44 (4.2-4.63)
Lot 2	0.2 (0-0.4)	0.26 (0.19-0.34)	12.8 (8.58-17.02)	15.49 (14.55-16.35)	25.3 (16.96-33.64)	28.48 (26.5-29.85)	2.98 (1.2-4.76)	4.12 (3.97-4.26)
Lot 3	0.2 (0-0.4)	0.28 (0.16-0.39)	7.72 (5.17-10.27)	7.57 (8.36-10.22)	22.1 (14.80-29.40)	24.74 (23.02-26.26)	2.87 (1.2-4.54)	3.96 (3.8-4.22)

The assays were performed in five replicates on five consecutive days

Ethical clearance
The institutional review board approved the study via reference number MICR No: 1427:2022.

Results

Accuracy verification
Accuracy was verified by testing multiple controls, including kit-controls, archived known-positive samples, third-party controls, and NIBSC controls. For all controls, both "actual" and "expected" results (and S/Co, if applicable) were concordant and verified the accuracy of the immunoassay [Table 1].

Mean values of three different kit-controls and one third-party control p24 antigen performed in five replicates on 5 consecutive days were acceptable and within the range defined by the manufacturer [Table 2].

Precision verification
Reproducibility of the HIV Combo assay was evaluated by doing both intra- and inter-day precision as per NCCLS guidelines EP15A-3 using controls from a single lot. "Actual" results showed excellent reproducibility of immunoassay. Both intra-day and inter-day precision showed acceptable values in terms of mean, SD, and %CV. The intra-day CV was slightly lower than inter-day CV [Table 3].

Limit of detection
The LOD was performed for HIV p24 antigen only. The p24 antigen concentration of 100 IU/mL, 10 IU/mL, 1 IU/mL, and 0.1 IU/mL were reactive. It was only at a concentration of 0.01 IU/ml (dilution 1:100,000) that the test returned nonreactive [Table 4]. The reactive result at a concentration of 0.1 IU/ml was better than the manufacturer's claim of LOD >0.48 IU/ml.

Sample type
S/Co for all ten serum and plasma samples were comparable without any significant variation. This indicated that either serum or plasma sample type might be used for the immunoassay.

Cross-reactivity
No cross-reactivity was observed for all known positive samples, including HBsAg, anti-HCV antibody, anti-syphilis antibody, anti-CMV antibody (IgG) and anti-SARS CoV2 IgG antibody, tested with the immunoassay. Furthermore, the HIV-2 p26 antigen was also nonreactive.

Interference
Both known positive and negative samples spiked with endogenous substances (hemolyzed RBC, bilirubin, lipid) did not show any significant variation in S/Co value in immunoassay, indicating the absence of interference by endogenous substances.

Clinical performance
The overall rate of false positivity of the present study was 0.081% (0.205% for donor samples and 0.016% for patient samples) [Table 5]. The rate of false positivity for both the patient sample and donor sample matched the manufacturer's claim.[9]

Discussion

Need for screening
CDC, US recommends that everyone between the age 13 and 64 years should get screened for HIV at least once, as part of routine health care and that people at higher risk for HIV infection should get screened more often.[7] CDC also recommends that all pregnant women should get screened for HIV infection as early as the first trimester and repeat testing in the third trimester to prevent vertical transmission. By 2030, UNAIDS 95–95–95 targets envision that 95% of people living with HIV should know their HIV status, 95% of people who know their HIV-positive status should have access to treatment and 95% of people on treatment should have suppressed viral loads.[11] Both CDC and UNAIDS emphasize on early, routine, and accurate testing for HIV.

Early detection
Early detection of HIV infection remains the targeted goal of laboratory medicine. Technological developments led to the introduction of HIV fourth-generation

Table 3: Inter-day and intra-day precision verification of HIV Combo assay using controls from a single lot

Type of controls	Name of controls	Expected mean value	Intra-day precision		Inter-day precision	
			Mean (SD)	CV (%)	Mean (SD)	CV (%)
Kit control	Negative control	0.2 (0-0.4)	0.26 (0.04)	15.5	0.26 (0.05)	19.99
	Anti-HIV 1 positive control	12.8 (8.58-17.02)	12.53 (0.34)	2.71	12.53 (0.47)	3.78
	Anti-HIV 2 positive control	25.3 (16.96-33.64)	25.07 (0.41)	1.62	25.07 (0.78)	3.09
Third-party control	Negative control	0.3 (0.1-0.5)	0.31 (0.05)	17.86	0.31 (0.06)	20.78
	HIV p24 antigen control	3.02 (1.2-4.84)	4.44 (0.09)	2.07	4.44 (0.09)	2.13
	Anti-HIV 1 positive control	2.58 (1.2-3.96)	2.94 (0.07)	2.45	2.94 (0.11)	3.74
	Anti-HIV 2 positive control	2.6 (1.2-4.0)	2.44 (0.07)	2.88	2.44 (0.07)	3.06

The assays were performed in five replicates on five consecutive. CV=Co-efficient of variation, SD=Standard deviation

assay, which can detect the presence of both anti-HIV 1 and 2 antibodies as well as HIV-1 p24 antigen, thereby capable of early detection of HIV infection, approximately 7–11 days earlier.[12] CDC recommends using a fourth-generation assay for screening of HIV infection.[8]

Performance evaluation

The HIV Combo assay showed accurate and precise results when different types of controls (HIV-1 and-2 antibodies and HIV p24 antigens) including kit controls, archived known positive samples, third-party controls, and WHO/NIBSC international standards, were used. We observed concordant results with different control lots, which indicates excellent reproducibility. All positive and negative controls, including manufacturers and third-party controls, showed excellent precision with CV% of <5% (allowable limit = 20%) and CV% close to 20% (allowable limit = 50%), respectively. LOD at a concentration of 0.1 IU/ml was even better than the 0.48 IU/ml LOD as claimed by the manufacturer. S/Co value did not vary, irrespective of sample type (serum or plasma). There was no cross-reactivity with other TTI reactive samples, and no interference was detected with endogenous substances.

In the present study, the FP test results (0.081%; rate of false positivity) were within the limits defined by the manufacturer. Ideally a test kit should be 100% sensitive (no false negative [FN]) and 100% specific (no false positive [FP]). However, no commercially available test kit can provide 100% sensitive and specific test results. In the case of blood screening, the sensitivity of a test method is accorded higher importance than specificity since the primary function of blood center is to provide the safest possible blood components to patients. In this bargain, there are few FP test results in donor screening, which are inevitable. These FP test results stress donors by causing unnecessary anxiety and psychosocial issues. These FP test results also necessitate additional tests and follow-up visits, which costs time and money.

Table 4: Detection of the limit of HIV-1 p24 antigen for HIV combo assay

HIV-1 p24 Ag Control (WHO NIBSC 90/636)				
Assay	**Dilution**	**Concentration (IU/mL)**	**Result (S/Co)**	**Remarks**
HIV combo assay	10	100	61.9	Reactive
	100	10	37.2	
	1000	1	10.4	
	10,000	0.1*	1.03	
	100,000	0.01	0.31	Nonreactive

*Manufacturer's claim of lower LOD is >0.48 IU/mL. WHO=World Health Organization, NIBSC=National Institute for Biological Standards and Controls, S/Co=Sample to cut-off ratio, LOD: Limit of detection

Similar studies

Raanathan *et al*.[13] evaluated VITROS HIV Combo assay through precision verification and diagnostic accuracy assessment by adopting CLSI guidelines and reported that the repeatability CV% cerebrovascular reactivity of 2.35% and within lab CV% (CVWL) of 3.77% was well within the manufacturer's claim (σR-3%, σWL-4.8%) and was found to be within acceptable limits.

De Paschale *et al*.[14] evaluated VITROS HIV Combo assay and showed comparable performance to two other commercially available HIV assays based on HIV 3rd and 4th generation assays. They concluded that the VITROS HIV Combo assay is able to correctly identify both acute and established HIV infections independently of viremia and HIV subtype.

Contestable *et al*.[15] evaluated the sensitivity of VITROS HIV Combo assay using 20 commercially available seroconversion panels and compared obtained results against results from a commercially available fourth-generation HIV Combo assay. They reported that the VITROS HIV Combo assay showed comparable results in 17 out of 20 seroconversion panels, and in the remaining three panels, the VITROS HIV Combo assay detected one bleed earlier than the commercially available assay.

Conclusion

HIV Combo assay meets the requirements for its use as a screening assay for HIV infection based on the analytical and clinical performance of the assay.

Credit author statement

AKT: Conceptualization, methodology, and supervision. AKT, GA, KC: Writing-original draft preparation, writing-reviewing, and editing. SP, SM, NY, VK: Data-curation, visualization, investigation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Table 5: Clinical performance using routine donor and patient samples

Samples	Number of samples	Reactive, *n* (%)	Confirmed positive, *n* (%)	Rate of false reactivity, *n* (%)
Donor	59,950	238 (0.40)	115 (0.19)	123 (0.205)
Patient	115,225	807 (0.70)	788 (0.68)	19 (0.016)
Total	175,175	1045 (0.60)	903 (0.51)	142 (0.081)

References

1. National AIDS Control Organization & ICMR-National Institute of Medical Statistics. India HIV Estimates 2019: Report. New Delhi: NACO, Ministry of Health and Family Welfare, Government of India; 2020. Available from: http://naco.gov.in/sites/default/files/INDIA%20HIV%20ESTIMATES.pdf. [Last accessed on 2021 Oct 04].
2. National AIDS Control Organization & ICMR-National Institute of Medical Statistics. HIV Estimations 2017: Technical Report. New Delhi: NACO, Ministry of Health and Family Welfare, Government of India; 2018. Available from: http://naco.gov.in/sites/default/files/HIV%20Estimations%202017%20Report_1.pdf. [Last accessed on 2021 Oct 04].
3. Sinha S, Dar L, Sharma SK. The common mode of transmission of HIV infection and common OIS at voluntary counseling and testing center (VCTC) at tertiary care hospital in North India. Chest 2007;132:643B.
4. Shaw GM, Hunter E. HIV transmission. Cold Spring Harb Perspect Med 2012;2:a006965.
5. Saran RK, editor. Transfusion Medicine: Technical Manual. 2nd ed. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2003.
6. Brust S, Duttmann H, Feldner J, Gürtler L, Thorstensson R, Simon F. Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV-1/2/O screening test. J Virol Methods 2000;90:153-65.
7. Branson BM., Owen SM, Wesolowski LG, Bennett B, Werner BG, Wroblewski KE, *et al*. Centers for Disease Control and Prevention (U.S.);Association of Public Health Laboratories.;National Center for HIV/AIDS, Viral Hepatitis, and TB Prevention (U.S.). Division of HIV/AIDS. Laboratory testing for the diagnosis of HIV infection : updated recommendations. 2014. Available from: https://stacks.cdc.gov/view/cdc/23447. [Last accessed on 2021 Oct 04].
8. CDC 2018 Quick Reference Guide: Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens. Available from: (https://stacks.cdc.gov/view/cdc/50872). [Last accessed on 2021 Oct 04].
9. VITROS HIV Combo Assay, Manufactured by Ortho Clinical Diagnostics. Instructions for Use; 2018. Version 2.0. Available from: https://www.fda.gov/media/118503/download. [Last accessed on 2022 Nov 01].
10. Clinical and Laboratory Standards Institute. User Verification of Precision and Estimation of Bias; Approved guidelines. 3rd ed. CLSI document EP15-A3. Wayne PA: Clinical and Laboratory Standards Institute; 2014. Available from: https://clsi.org/media/1431/ep15a3_sample.pdf. [Last accessed on 2021 Oct 04].
11. UNAIDS 2015. UNAIDS Joint United Nations Programme on HIV/AIDS. Available from: https://www.unaids.org/sites/default/files/media_asset/201506_JC2743_Understanding_FastTrack_en.pdf. [Last accessed on 2021 Oct 04].
12. Mitchell EO, Stewart G, Bajzik O, Ferret M, Bentsen C, Shriver MK. Performance comparison of the 4th generation BIO-RAD laboratories GS HIV Combo Ag/Ab EIA on the EVOLIS™ automated system versus Abbott ARCHITECT HIV Ag/Ab Combo, Ortho Anti-HIV 1+2 EIA on Vitros ECi and Siemens HIV-1/O/2 enhanced on Advia Centaur. J Clin Virol 2013;58 Suppl 1:e79-84.
13. Raanathan S, Chakravarthy S, Smitha S, Nallathambi T. Precision and diagnostic accuracy verification of VITROS HIV combo assay. Internet J Rheumatol Clin Immunol 2020;13;7.
14. De Paschale M, Manco MT, Cianflone A, Belvisi L, Cagnin D, Cerulli T, *et al.* Evaluation of the vitros hiv combo 4th generation test for the identification of HIV infections. J Clin Virol 2018;108:77-82.
15. Contestable P, Hirsch B, Gardner A. An evaluation of performance of the VITROS® immunodiagnostic products Anti-SARS-CoV-2 IgG quantitative assay. Transfusion 2021. p. 174A-5A.