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Is commercial control necessary as a third party internal control for HIV – antibody ELISA testing? A comparative study in NABL accredited laboratory

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Abstract

Aims & Objectives: To assess and compare the feasibility, availability, affordability, stability of In-house and commercial third party internal control of HIV antibody ELISA test.

Methods: A comparative study was conducted in NABL Accredited Diagnostic Laboratory at Krishna Institute of Medical Sciences Karad during June 2014 to July 2016. Sample size includes total of 457 ELISA tests which were carried out on 11425 patients along with internal, external commercial and in-house control as per NACO guidelines. Descriptive and inferential statistical tests were applied to find out significant difference between in-house and commercial control.

Results: There is significant difference in mean values of positive kit control and commercial positive control ($p<0.05$). There is also significant difference observed in mean values of commercial positive control and in-house positive control ($p<0.05$). The coefficient variance of positive in -house control is better (3.32) than commercial positive control (4.60) which indicates, in-house control is more precise than commercial control. The kit cut off values remains constant throughout the month indicates best technical competence, less environmental influences and reagent stability. The average cost of screening for HIV with ELISA and in-house control was Rs. 57/ per person.

Conclusion: There is no significant difference in mean values of positive kit control and in-house positive control, the diagnostic cost, feasibility and percentage CV% is better observed in in-house control as compared to commercial control indicating in-house control could be considered as third party internal control for HIV antibody ELISA test.

Keywords: In-house control, Third party internal controls, Commercial controls, ELISA test

Introduction

Human immunodeficiency viruses (HIV) belong to the family Retroviridae and subfamily Lentivirinae and Genus Lentivirus. There are two types of HIV are recognized; HIV-1 and HIV-2 and both differ in geographical distribution, biological and molecular characteristics and extent of transmissibility.(1) According to current global HIV statistics, HIV continues to be a major global public health issue reflecting both developed as well as developing countries. In 2015, an estimated 36.7 million people were living with HIV (including 1.8 million children) – a global HIV prevalence of 0.8% and majority of them live in low- and middle- income countries with 1.1 million people died of AIDS-related illnesses.(2) India has the third largest HIV epidemic in the world. In 2013, HIV prevalence in India was estimated to 0.3% with large geographical variations. The five states with the highest HIV prevalence (Nagaland, Mizoram, Manipur, Andhra Pradesh and Karnataka) are in the south or east of the country. Some states in the north and northeast of the country, report rising HIV prevalence. Overall, India's HIV epidemic is slowing down, with a 19% decline in new HIV infections(130,000 in 2013), and a 38% decline in AIDS-related deaths between 2005 and 2013.(3)

Gold standard technologies with high sensitivity and specificity are used worldwide to efficiently diagnose and monitor HIV infection. Enzyme-linked immunosorbent assays (ELISAs) and simple/rapid tests are performed to diagnose HIV infection by antibody detection. Flow cytometry is used to monitor CD4+ T-cell count and thereby to determine initiation of ART. The more expensive nucleic acid tests, such as polymerase chain reaction (PCR) assays, are used for monitoring the HIV load.(4) Enzyme-linked immunosorbent assays (ELISA) was developed independently and simultaneously by the research group of Peter Perlmann and Eva Engvall at Stockholm University in Sweden and by the research group of Anton Schuurs and Bauke van Weemen in The Netherlands.(5) Today, fully automated instruments in medical laboratories around the world use the immunoassay principle with an enzyme as the reporter label for routine measurements of innumerable analytes in patient samples. ELISA has become household names for medical laboratories, manufacturers of in vitro diagnostic products, regulatory bodies, and external quality assessment and proficiency-testing organizations.

As per NABL document, 112 laboratory are classified as Small Laboratory: A laboratory receiving up to 100 patients per day, Medium Laboratory: A laboratory receiving up to 101-400 patients per day, Large Laboratory: A laboratory receiving above 400 patients per day, and all laboratory have to maintain quality control (QC) and quality assurance(QA) in HIV ELISA testing. To achieve QC and maintain QA,

NABL recommended using internal control, external third party control, during each ELISA test.[6,7] The term "third party" is used to describe a quality control product that helps provide an independent assessment of a diagnostic device or method, and is not optimized for any specific instrument or reagent system. Third party controls are manufactured independently of the test system calibrators and reagents. Such controls generally begin with a human base matrix that helps provide a product more similar to a patient sample. Third party controls with a longer shelf life allow use of the same control lot over multiple changes in reagents and calibrators, giving the laboratory the ability to detect shifts that may occur with new reagents or calibrators.[8] In our laboratory along with these controls we have also used in –house controls in each ELISA test.

Study aimed to recommend that as there are limited number of companies which provides commercial controls, having high diagnostic cost, low open vial stability and low practice. We took an observational comparative study to assess the affordability, availability, quality, validity, open vial shelf-life, of commercial control and in-house control. As in house control is easy to prepare, with low cost, accessible with longer stability and utilization of in-house control could reduce the economic burden on patient, laboratory logistics, increase access by remote laboratories in developing countries with diagnostic accuracy and patient satisfaction.

Material and Methods

An observational comparative study was done in NABL Accredited KIMS Diagnostic Laboratory in Krishna Institute of Medical Sciences Deemed University, Karad. The study period was from June 2014 to July 2016. Total of 457 HIV Antibody detection ELISA tests were carried out on 11425 samples. Along with each ELISA test run both (commercial and In-house controls) were tested during the study period.

ELISA test was run on samples collected from patients along with internal control, external commercial controls and in-house controls after a written consent as per NACO guidelines. The blood was allowed to clot for 30 minutes and then centrifuged at 2000 to 3000 revolutions per minute (rpm) for ten minutes to separate the serum. The serum was separated and refrigerated. The serum was aliquoted in pre-labelled screw-capped, sterile storage vials using micropipette tips for testing/storage. No preservatives added as they interfere with the testing. Commercially available ELISA kit was used to detect antibodies to HIV 1 and HIV2. It is a third generation solid phase Enzyme Linked immunosorbent Assay (ELISA) which employs highly purified recombinant antigens representing envelope glycoprotein gp41 and core p24-o subtype fusion polypeptide of HIV-1 and envelope glycoprotein gp 36 of HIV -2.

Commercial available controls in the market (positive control and negative control) were used in each run of ELISA. Patient's anonymity was maintained and in- house positive control was prepared by selecting sero- positive serum (only for HIV antibody) samples of different patients with OD value above 2. 000. HIV Sero-positive serum reactive for Hepatitis B (positive for HBsAg), and antibody body to HCV were excluded while preparing of in- house positive controls of HIV. Negative in- house HIV controls prepared by selecting serum which has absorbance value below cut off and not in gray zone (i.e. below 0.0). These sera were retested with another ELISA kit and supplementary Rapid test kit of two different principles and also by inter-laboratory testing from referral laboratories enrolled in EQAS.

Borderline reactor was also prepared from sero-positive serum samples of patients. After collection of serum it was heat inactivated at 56°C for 30 min. Serial dilutions of these positive sera done with a sero-negative serum. Serial dilutions 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000 were prepared and ELISA was run with each dilution. Serial dilution 1:8000 showing result near to cut off result of ELISA test run was selected as the borderline reactor. Aliquots of these borderline reactor samples were stored at -20°C deep freezer for 1 year. The aliquot in use during each ELISA test was maintained at 2-8^{0}C for 1 week.[9] ELISA procedure was carried out as per manufacturer instructions and absorbance was measured for each well at 450 nm with ELISA reader. NACO algorithm was followed for positive sera while testing and interpretation of ELISA test reports. Quantity of commercial control (100ul), in-house control (100ul) and borderline control (100ul) were used as per manufacturer instructions. ELISA results were interpreted on OD values, ER ratio values were used to determine mean, SD, and %CV of commercial controls and in –house controls.

Inclusion criteria: All valid ELISA tests conducted during study period.

Exclusion criteria: Invalid ELISA test as per manufactures instructions. Serum with OD absorbance value 2 and above was selected for preparation of positive in-house control, and OD below O.O was used for negative control. Rest will be excluded while preparation of control.

Statistical analysis: Descriptive and inferential statistical tests were applied to find out significant difference between in-house and commercial control

Ethical consideration, Confidentiality and Anonymity: IERC was obtained, Patients name, laboratory details were well maintained and kept in office of principal investigator.

Results

Table 1: Comparison of mean values of positive kit control, commercial positive control and in-house positive control

Variables	Positive kit control	Commercial positive control	In- house positive control
Mean	2.76	2.73	2.76
SD	0.05	0.06	0.20
SD Error	0.01	0.01	0.03
P value@	0.01*		0.94$
T@	2.58		0.07$
95% CI@	0.006 to 0.005		0.06 to 0.07$
P value #	0.042		
T#	0.80		
95% CI#	-0.10 to 0.04		

(@: values of positive kit control and commercial positive control, #: values in-house control and commercial positive control and $: values of positive kit control and in-house positive control, *: significant p value at 95% CI.)

There is significant difference in mean values of positive kit control and commercial positive control ($p<0.05$). There is no significant difference in mean values of positive kit control and in-house positive control ($p>0.05$). There is significant difference in mean values in-house control and commercial positive control ($p<0.05$) (Table 1).

Table 2: Comparison of mean values of negative kit control, commercial negative control and in house control

Variables	Negative kit control	Commercial negative control	In- house negative control
Mean	0.028	0.034	0.03
SD	0.008	0.01	0.01
SD Error	0.001	0.002	0.002
P value@	0.0001*		0.001*$
T@	4.91		3.54$
95%CI@	-0.008 to –0.003		-0.007 to – 0.002$
P value#	0.39		
T#	0.86		
95%CI#	0.06 to 0.07		

(@: values of negative kit control and commercial negative control, #: values in-house control and commercial negative control and $: values of negative kit control and in-house negative control)

There is significant difference between mean values of negative kit control and commercial negative control ($P<0.05$). There is significant difference between mean values of negative kit control and in-house control ($p<0.05$). There is no significant difference in commercial negative and in-house negative control ($p >0.05$) (Table 2).

Table 3: Comparison of affordability, feasibility and stability of commercial control and in-house control

Variables	Commercial control	In-house control
Cost	Rs. 55 per Test	Free of cost
Feasibility	Few suppliers not possible to prepare it in laboratory	Feasible to prepare it in laboratory
Shelf life at 2-8^{0}C	3 years	1-2 year
Open vial stability at 2-8^{0}C	60 days	100 days
Availability	Not easily available	Easily available

The present study conducted in NABL accredited rural tertiary care hospital depict the average cost of commercial control is Rs.55 per test, while in-house it is free of cost. Shelf –life of commercial control is 3 years, while for in-house control is of 1-2 year. In-house control open vial stability is more as compared to commercial controls. The average cost for HIV-antibody screening by ELISA test with the commercial control is Rs. 112/ per person, whereas it is only Rs. 57/ per person with the in-house control (Table 3).

Table 4: Comparison of laboratory %CV of Positive kit control, Commercial positive control, in-house positive control and borderline reactor control per month

Months	Positive Control (kit)	Commercial Positive control	Borderline Reactor In- house	In-house Positive control
June 14	6.272781	4.144288	22.38922	5.238091
July 14	1.021333	2.888923	17.7279	1.422161
Aug 14	2.360136	2.452732	29.55398	1.641513
Sept 14	0.359079	1.14094	19.5945	1.11976
Oct 14	0.801169	1.427305	16.30858	1.080746
Nov 14	1.863085	2.99432	32.67676	2.236878
Dec 14	2.733541	1.900031	21.10474	3.268299
Jan 15	0.992396	0.90079	16.10547	1.030345
Feb	1.180498	2.293116	20.09137	1.294448
Marc	2.733541	1.900031	21.10473	3.268299
April	2.192385	2.279664	10.37613	2.44205
May	0.947152	1.215745	7.826874	1.717952
June	0.656622	1.036113	13.93844	1.439426
July	7.20917	2.97908	12.36312	2.806252
Aug	2.24374	3.140534	12.8256	19.81374
Sept	3.767338	4.687434	12.6504	4.538317
Oct	2.231829	2.026829	19.19853	2.023007
Nov	1.507195	2.227472	21.13783	2.135252
Dec	2.704542	4.905546	12.90749	2.055142
16-Jan	1.26733	5.460621	15.7899	2.070804
Feb	1.598019	2.115481	12.20392	5.097905
March	2.884001	4.914419	8.329443	4.320087
April	2.35823	5.102447	14.19841	5.59166
May	2.181869	28.66233	7.411601	3.730255
CV% mean (2yr & 1month)	2.209944	3.866506	17.10022	3.390933

The above table shows CV% per month for each control. As per NABL requirement% CV required for quality control must be around 10, which are shown by kit control, commercial control and in- control. Borderline reactor shows average laboratory % CV towards a higher side as it was prepared from reactive serum which was diluted in non –reactive sera. This may be the reason for the variation The coefficient variance of positive in -house control is better (3.32) than commercial positive control (4.60) which indicates, in-house control is more precise than commercial control.

Table 5: Laboratory Mean cut-off per month per year

2014-2015		2015-2016	
Month	**Mean cut-off**	**Month**	**Mean cut-off**
June	0.224222	June	0.219211
July	0.225647	July	0.233889
Aug	0.235889	Aug	0.216130
Sep	0.229211	Sep	0.219789
Oct	0.221769	Oct	0.217818
Nov	0.238000	Nov	0.226563
Dec	0.224600	Dec	0.229471
Jan	0.221077	Jan	0.216368
Feb	0.22106	Feb	0.221077
March	0.230046	March	0.223591
April	0.233200	April	0.230217
May	0.223353	May	0.230391
Mean	0.2273395	Mean	0.223710
Laboratory means cut-off (of 2 year): 0.225313			

This Table 5 shows, average cut off values of each month and laboratory mean cut off of two years. When daily cut-off values compared in each month it was almost same, and when mean cut-off values observed per month over a period of two years, monthly cut-off was also almost same during June 14 to May16.

Discussion

ELISA test is commonly used in many laboratories for HIV screening as a semi quantitative test. NABL recommends third party control for quantitative tests. HIV is pandemic infection so it is possible to have third party control, but there are other diseases which are not

pandemic, some may be seasonal in such cases third party control may not available all the times. Our study results highlighted few comparative points which will help to formulate policy regarding use of in-house control as one of the quality control indictor in small laboratories to maintain quality control and quality assurance in testing.

Present study showed that there is significant difference in mean values of positive kit control and commercial positive control and difference could be due to selection of reactive serum from patient which was tested reactivity by kits which uses different principles. However; there is no significant difference reported with in- house positive control mainly due to selection of reactive sample from same diagnostic kit. There is significant difference in mean values of commercial positive and in- house positive control and this difference could be due to selection of single reactive (only reactive for HIV antibody) sera in in-house control whereas, in commercial control, more than one antibody sera was used. The similar observations are not yet been reported by anybody and anywhere, so this is the first documentary evidence on utilization of commercial and in house positive control.

In context to negative controls there is significant difference between mean values of negative kit control and commercial as well as in-house negative control; however there is no significant difference reported in mean values of commercial and in-house negative controls. The coefficient of variance of positive in-house control is better than commercial positive control which indicates that in-house control is more precise. The daily cut-off values compared in each month was almost same, but when mean cut-off values observed per month over a period of two years ,monthly cut-off was also almost same with few variation during June 14 to July16 this could be due change of lot of kits.

Accordingly, screening tests possess a high degree of sensitivity, whereas confirmatory assays have a high specificity. Tests with high sensitivity produce few false-negative results, whereas tests with high specificity produce few false-positive results. These classes of assays, performed in tandem, produce results that are highly accurate, reliable, and appropriate to protect the blood supply or assist in the diagnosis of HIV infection. Technical errors do occur, however, and there are biologic factors that can limit the accuracy of HIV tests. Therefore, along with the testing process, there is the requirement for an extraordinary and dedicated quality assurance program. ELISA is the most commonly used type of test to screen for HIV infection because of its relatively simple methodology, inherent high sensitivity, and suitability for testing large numbers of samples, particularly in blood testing centers.[10] The concept of laboratory accreditation was developed to provide third-party certification that a laboratory is competent to perform the specific test or type of tests. Laboratory accreditation is a means to improve customer confidence in the test reports issued by the laboratory so that the clinicians and through them the patients shall accept the reports with confidence. It provides feedback to laboratories as to whether they are performing their work in accordance with international criteria for technical competence.[11] As per NABL guidelines ISO 15189:2012 it is necessary to maintain the Quality indicator before testing (pre-analytical), during testing (analytical), post analytical testing. These third party control (commercial and in- house control) will determine the overall performance of assay, accuracy, and precision of the ELISA test. The commercial positive control used in study is unassayed and was prepared from human plasma or serum reactive for antibody to HIV, antibody to HCV, antibody to HTLV-I, HBsAg, antibody to HBc and antibody to CMV, proteins from human and bovine sources,, antimicrobial agents as preservative, and stabilizers. It has shelf life 3 year and open vial stability 60 days at 2-8^{0}C. The commercial negative control is processed human plasma or serum from human sources with same shelf life and open vial stability as that of positive control. Our study in-house positive control prepared from reactive patient serum and pooled serum from HIV reactive donors blood, which was non- reactive for HBsAg, antibody to HCV. The documentary evidence suggested that HIV test serum samples are stable for 2 to18 years and they also showed reproducible results by ELISA/EIA when stored at -20^{0}c.[12] There are many causes of false positive reactions to HIV antibody ELISA test, few to list like: Anti-carbohydrate antibodies, Naturally-occurring antibodies, Passive immunization: receipt of gamma globulin or immune globulin as prophylaxis against infection which contains antibodies, Leprosy, Tuberculosis, Mycobacterium avium, Systemic lupus erythematous, Renal (kidney) failure, Hemodialysis/ renal failure, Alpha interferon therapy in hemodialysis patients, Flu, Flu vaccination, Herpes simplex I, Herpes simplex II, Recent viral infection or exposure to viral vaccines, Pregnancy in multiparous women, Malaria Hypergammaglobulinemia (high levels of antibodies) etc. This may be due to cross reactivity of antigens to non-HIV antibodies. It is known that cross reacting antibody may affect specificity of control; the control with single antibody may be more specific and can be used in test, then the control having more than one antibody. In present literature we did not find out any comparative study between commercial controls and in-house controls.

Conclusion

There is no significant difference in mean values of positive kit control and in-house positive control, the diagnostic cost, feasibility and percentage CV% is better observed in in-house control as compared to commercial control indicating in-house control could be

considered as third party internal control for HIV antibody ELISA test.

Perspective of the study: laboratory, patient and community: When ELISA tests used for immunodiagnostic of various microbial infection (bacterial, viral, fungal, parasitic) diseases, and such test has to include in a scope of laboratory to accreditation by NABL many a time there is difficulty to get a reference control from reference laboratory and commercial company. Even if they are available in market, it is beyond the reach of small laboratories situated in peripheral regions of developing countries. Considering all the facts and situations, in- house controls are feasible, cost-effective and can be recommended. This will reduce diagnostic cost which ultimately beneficial to patient. In developing country even small laboratories can afford this in-house control and will used daily after getting validated it from accredited laboratories. That will help to increase their number of tests in scope of laboratories.

Recommendation

1. In- house controls are easily available, easy to prepare, cost effective, and specificity as well as sensitivity is similar to commercial control it can be used in peripheral laboratory for quality control.
2. ELISA Tests is run on batch of sera and when same kit of same lot is used, and if cut off value is the same with no much variation, in such situations in- house control can be used daily during test and commercial control is added only at the time of change in lot of kit. That will reduce the cost of test and ultimately it will be beneficial for small laboratories and stakeholders.
3. Large laboratory can afford commercial control as their inflow of patient is high but small laboratory cannot, as they are situated at remote places and inflow of patient is less.

Future plan

Currently used controls (commercial and in-house) both are unassay so our future plan use to quantitate the HIV antibodies from patient sera which will help to manufacture / develop the assayed control.

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