Research Article

Achievement of quality standards in HIV testing of a State Reference Laboratory of North India through External Quality Assurance Scheme (EQAS): an evaluation of seven years' experience

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Abstract

Background: Centre for HIV Testing, Lady Hardinge Medical College (CHT-LHMC) is working as a State Reference Laboratory (SRL) from 2007 and regularly performing External Quality Assurance Scheme (EQAS) activities of 24 Integrated Counselling and Testing Centres (ICTCs) and 16 blood banks (BBs) linked to it.

Objective: To evaluate and analyze data of 7 years (2007–2013) related to the EQAS activities of SRL (i.e., proficiency testing and retesting).

Materials and Methods: It was a retrospective study. Proficiency tests were carried out twice a year from 2007–2013. The samples from BBs for EQAS retesting were tested using third-generation ELISA kits and samples from ICTCs by the kits provided by Delhi State AIDS Control Society.

Results: A total of 8194 (4992 samples from BBs and 3202 samples from ICTCs) samples were received over a period of 7 years for EQAS retesting. HIV-positive samples from BB and ICTCs were 163 (3.26%) and 646 (20.17%), respectively. Nineteen (0.38%) were tested as positive discordant and 3 (0.06%) were tested as negative discordant out of 4992 samples received from the BBs. No indeterminate result was found among the samples from ICTCs. Proficiency testing showed continuous improvement over the years with 100% participation in years 2012 and 2013. The various attributing factors affecting the test results as well as nonparticipation in proficiency testing were evaluated and discussed.

Conclusion: CHT-SRL-LHMC is a laboratory accredited by National Accreditation Board for Testing and Calibration Laboratories, and through EQAS activities the laboratory is providing constant support and opportunities for improvement of its linked centers.

KEY WORDS: State Reference Laboratory, HIV, External Quality Assurance Scheme, proficiency testing, retesting

Introduction

Quality assurance (QA) refers to planned, step-by-step activities that let one know that the testing is being carried out correctly, results are accurate, and mistakes are found and corrected to avoid adverse outcome.[1] To achieve high-quality result, all laboratory personnel in a testing network should be aware of the procedures for quality performance.[2] According to the ISO definition, External Quality Assessment Scheme (EQAS) refers to a system of objectively checking laboratory results by means of an external agency, including comparison of a laboratory's result at intervals with those of other laboratories, the main objective being the establishment of trueness.[3] QA is an integral part of serodiagnosis of HIV as the tests performed to detect the antibodies against HIV depend on the quality of the conditions under which the tests are performed, and thereby, there is always a possibility of false-positive or

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false-negative results. Therefore, consistent reproduction of reliable test results requires properly maintained QA activities to control the technical conditions related to performance of the HIV diagnostic tests.

Two important methods used in EQAS are proficiency testing (PT) and retesting.

Proficiency testing as per Clinical and Laboratory Standards Institute guidelines is "A program in which multiple samples are periodically sent to members of a group of laboratories for analysis and/or identification; whereby each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratories and others." It is the most commonly used type of EQA as it is able to address many laboratory methods. In a typical PT program, challenge samples are provided at regular intervals. An optimal frequency will be 3–4 times yearly. If the program cannot provide challenges with this frequency, the laboratory may be able to seek additional sources. The laboratories participating in the program analyze the samples and return their results to the central organization. Results are evaluated and analyzed, and the laboratories are provided with information about their performance and how they compared with other participants. The participating laboratories use the information regarding their performance to make appropriate changes and improvements.[4]

Retesting is a program where samples analyzed are retested, allowing for interlaboratory comparison. It is an important method in the EQAS of HIV testing. HIV rapid testing presents some special challenges, because it is often performed outside a traditional laboratory and the kits are single-use and cannot be subjected to the usual quality control methods that laboratories use. Therefore, retesting of some of the samples using a different process such as enzyme immunoassay (EIA) or ELISA (enzyme-linked immunosorbent assay) helps to assess the quality of the original testing. Process of retesting is performed by a reference laboratory to ensure quality and it is not processed as a blinded process as it is unnecessary.[5]

Centre for HIV Testing Lady Hardinge Medical College (CHT-LHMC) is working as a State Reference Laboratory (SRL) from the year 2007. There are 24 Integrated Counselling and Testing Centres (ICTCs) and 16 blood banks (10 private and 6 government blood banks) linked to CHT-SRL LHMC. CHT-SRL-LHMC conducts EQAS programs as well as coordinate its linked ICTCs and blood banks (BBs) to National Reference Centre and conduct regular training programs in collaboration with Delhi State AIDS Control Society (DSACS) to constantly improve and maintain standard of the quality of HIV testing done in this centers.

Aims and Objectives

This study was undertaken to retrospectively evaluate the activities related to EQAS, which include retesting and proficiency testing of linked ICTCs and BBs of SRL from 2007 to 2013.

Materials and Methods

The following activities for QA of HIV testing were performed as per the NACO guidelines.[6]

EQAS Proficiency Testing

Proficiency testing was not conducted in the year 2007 and 2008, as no panel sera was received from National Reference Laboratory (NRL). In the year 2009, there was only one round of proficiency testing as SRL had received the panel sera from the NRL only once, that is, in the month of August 2009. It was in the year 2010 that three rounds of proficiency testing were conducted. Thereafter, the proficiency testing is being conducted twice a year. Only the linked ICTCs participated in the proficiency testing, not the BBs. For proficiency testing, four-member panel sera received from NRL were divided in aliquots and distributed to each linked ICTC within 7 days of receiving of panel sera from NRL. Representative from ICTCs come to SRL for collection of proficiency testing samples. The ICTCs had to perform and send the results of the proficiency testing samples to SRL within 7 working days from the date of receiving of proficiency testing samples from the SRL. The SRL compiled the results of proficiency testing of all the linked ICTCs and the compiled report was sent to the NRL within another 7 days of receiving of reports from the linked ICTCs. Any discordant test results were informed to the respective centers.

EQAS Retesting

Serum samples were received from linked ICTCs and BBs from 2007 to 2013 for retesting. The process of retesting started in the year 2007 with three ICTCs and seven BBs. These linked centers used to send samples for retesting every month and the number of samples sent were 5% of total samples tested in that particular month. This process continued till end of 2008. From 2009, the process was streamlined and samples were received on quarterly basis in the months of January, April, July, and October for retesting. The linked centers sent 20% positive and 5% negative of the total samples collected and tested in first 7 working days of each quarter for retesting.

Samples from ICTCs for EQAS retesting were tested by the three rapid test kits. The principles of the kits were immunochromatography, immunoconcentration, and ELISA based. The kits provided by DSACS used to vary, depending on the availability of the kits, but while performing the rapid testing of HIV at any point of time the following rules were followed: (1) the first rapid test kit should have high sensitivity and the rest two rapid test kits should have high specificity. (2) The three kits should be of three principles and should have different antigen preparations.

The samples for retesting from BBs were tested using third-generation ELISA kits.

Sample rejection criteria were insufficient and hemolyzed samples, samples not properly labels, and samples not properly sent (three-layer packaging). The results of the samples

retested were sent back to respective centers within 7 days (turnaround time) of receipt of samples. Few terminologies used in EQAS retesting are as follows:

Indeterminate result: When a result of the same sample is different in ICTC and SRL the sample is termed as indeterminate.

Discordant result: This term is used for testing of BB EQAS retesting samples. A sample tested positive for HIV in BB and negative in SRL is termed as positive discordant (PD) and a sample tested negative at BB and positive at SRL is termed as negative discordant (ND) sample.

Ethical Clearance

This study was based on retrospective collection and analysis of EQAS activities, which are regularly conducted by SRL-LHMC under the approval and guidance of National AIDS Control Organization (NACO), India, under National AIDS Control Program.

Results

Results of Proficiency Testing

The participation was lowest (40%) in the first round of proficiency testing in 2011, which showed improvement (100%) in the second round of proficiency testing in the same year itself. The detail results of participation of linked ICTCs in proficiency testing are shown in Table 1.

Table 1: Result of proficiency testing of linked ICTCs

Years	1st round	2nd round	3rd round
2009	84.6% (11/13)#	–	–
2010	100% (23/23)#	95.6% (22/23)#	88% (22/25)#
2011	40% (10/22)#	100% (22/22)#	–
2012	100% (22/22)#	100% (22/22)#	–
2013	100% (23/23)#	95.8% (23/24)#	–

#Total number of ICTCs participated in the proficiency testing/total number of linked ICTCs at that point of time.

Results of EQAS Retesting

Blood Banks

A total of 4992 samples were received by SRL-CHT-LHMC from 16 linked BBs for EQAS retesting in the past 7 years (2007–2013). Among them, 163 (3.27%) were HIV-positive and 4822 (96.59%) were HIV negative; and 22 (0.44%) samples reported discordant results. Nineteen (0.38%) samples were PD and 3 (0.06%) samples were ND [Table 2; Figure 1].

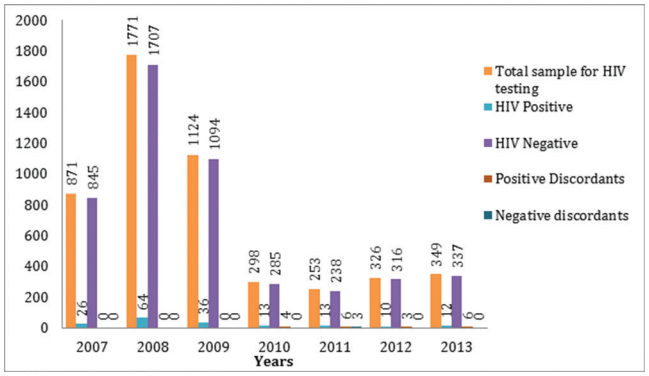

Figure 1: Result of EQAS retesting of linked blood banks.

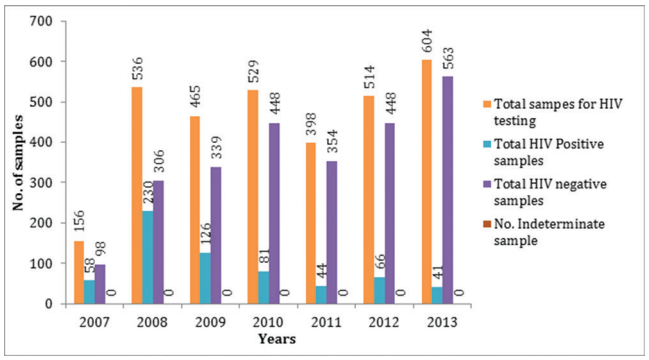

Figure 2: Result of EQAS retesting of ICTCs (2007–2013).

Table 2: Result of EQAS retesting of linked blood banks

Year	Total sample for HIV testing	HIV positive	HIV negative	Negative discordant	Positive discordant
2007	871	26	845	0	0
2008	1771	64	1707	0	0
2009	1124	36	1094	0	0
2010	298	13	285	0	4
2011	253	13	238	3	6
2012	326	10	316	0	3
2013	349	12	337	0	6
Total	4992	163	4822	3#	19

#Negative discordant samples: 2 samples (January): first sample (BB tested negative, SRL tested positive, NRL confirmed negative), second sample (BB tested negative, SRL tested positive, NRL confirmed negative), third sample (July) (BB tested negative, SRL tested positive, NRL confirmed negative).

Table 3: Result of EQAS retesting of ICTCs

Years	Total samples for HIV testing	Total HIV-positive samples	Total HIV-negative samples	No. indeterminate sample
2007	156	58	98	0
2008	536	230	306	0
2009	465	126	339	0
2010	529	81	448	0
2011	398	44	354	0
2012	514	66	448	0
2013	604	41	563	0
Total	3202	646	2556	0

Table 4: Comparison of results of EQAS retesting of blood banks and ICTCs

Linked center	HIV		Total no of samples tested for HIV	No. of samples giving discordant/indeterminate results		
	Positive	Negative		Positive discordant	Negative discordant	Indeterminate
Blood banks (total no: 16)	163	4822	4992	19	3	–
ICTC (total no: 24)	646	2556	3202	–	–	0

ICTC

A total of 3202 were received from the_linked ICTCs for EQAS retesting. Among them, 646 (20.17%) samples were HIV positive and 2556 (79.83%) samples were HIV negative. No sample was found to show indeterminate result [Table 3; Figure 2]. The results of EQAS retesting of BBs and ICTC are summarized and compared in Table 4.

Discussion

The critical points to the success of HIV and AIDS programs are the accuracy and reliability of diagnostics and clinical monitoring of tests performed. With the increasing disease burden, the expanding HIV testing in developing countries like India has not kept pace with QA programs to monitor the performance of these tests, which raises concerns about test accuracy. To ensure reliability and reduce errors to a minimum, it is essential to implement a quality system in all laboratories and testing sites that addresses all aspects of testing in the laboratory.[3]

In India, the EQAS program has been implemented by NACO since 2000. It functions with Apex Laboratory and 13 NRL and thousands of subcenters covering the entire government medical colleges, hospitals , BBs, and primary health centers in each state. The EQAS program is linked with the respective State AIDS Control Society in each state for the effective functioning under the NACO.[2] The first report of EQAS was published by Belk and Sunderman in 1947.[7]

Discussion of EQAS Retesting Results

A total of 4992 samples from BBs and 3202 samples from ICTCs were tested in SRL-CHT-LHMC as a part of EQAS retesting over the period of 7 years (2007–2013). In the first 3 years, that is, 2007–2009, the BBs as well as ICTCs used to send samples for EQAS retesting every month, as described in the Materials and Method section. Therefore, the number of samples in that period for EQAS retesting were high compared to those received from 2010 to 2013. This fact is reflecting more in the EQAS retesting data of linked BBs. SRL-LHMC was successful in streamlining the process of EQAS from later part of 2009 by formulating an EQAS calendar regarding collection of samples for EQAS retesting. This EQAS calendar was dispatched to every linked center before starting of EQAS for that particular year. This process has helped the linked center to understand the process of retesting and send the samples for EQAS retesting in a systematic and easy manner.

Of 4992 samples from BBs for EQAS retesting, 22(0.44%) samples gave discordant results [19 (0.38%) PD and 3 (0.06%) ND]. The higher number of PD samples, that is, the sample tested positive for HIV in the BB and the same sample tested negative in SRL-LHMC during EQAS retesting, might be attributed to the use of different kits for retesting of samples in BBs and SRL-LHMC. The linked BBs were using fourth-generation ELISA kits and CHT-SRL-LHMC was using third-generation ELISA kits for confirmation. Similarly, the three samples whose results were found to be ND (sample tested negative in BB and same sample tested positive in SRL-LHMC) were sent to NRL for confirmation and the final result of all the three samples were given negative by NRL after conducting Western blot test. Moreover, the use of the above-mentioned ELISA kits in SRL-LHMC is subjected to change depending on the availability of the kits at that point of time. So, there is an urgent need of formulating a new policy for EQAS retesting of BBs or solving the above-mentioned discrepancies by uninterrupted supply of similar kits in BBs as well as SRL for proper comparison and confirmation of EQAS retesting results, thereby maintaining quality standards of HIV testing.

In case of EQAS retesting of ICTCs, not a single sample was tested indeterminate, that is, of 3202 samples tested over a period of 7 years, the results of the ICTCs and SRL were similar. This striking difference in results of EQAS retesting of BBs and ICTCs was because ICTCs are using Rapid Test kits provided by DSACS for testing and SRL is also using the same kits (in terms of principles) for confirmation of ICTC results. This result shows the importance of using similar kits in all levels of testing and confirmation to minimize the discrepancy of results.

Proficiency Testing

Data reflect that only linked ICTCs are participating in the proficiency testing, not the BBs. Participation in proficiency testing is a key component of any laboratory QA program, whether locally, nationally, or internationally.[4] So, there is a need of proper guidelines from NACO so as to encourage the participation of the BBs in the proficiency testing to monitor the standard of the HIV testing in the linked BBs.

SRL-LHMC started the proficiency testing with 13 linked ICTCs in 2009. Eleven ICTCs participated in the proficiency testing in that particular year. The reason of nonparticipation of rest two ICTCs was nonavailability of the HIV testing kits in those centers at that point of time. The lowest participation in proficiency testing was seen in 2011 when only 40% (10/22) ICTCs participated in the first round of proficiency testing. The reason of such low participation was also the same, that is, at 11 ICTCs at that point of time, the HIV testing kits were not available. Therefore, availability of HIV testing kits throughout the year is of utmost importance so that the linked ICTCs can participate in both the rounds of proficiency testing to check their quality standards. To ensure the availability of the kits, it is the responsibility of the concerned authority to provide them continuous supply of kits in adequate amount as well as the responsibility of the laboratory in charge of each linked center to maintain a proper stock register so as to inform the authority on time before they exhaust all the kits. From 2012, the participation in proficiency testing showed very encouraging results with high percentage of participation as mentioned in the Table 1. The linked ICTCs participating in this proficiency testing gave 100% concordant result from 2009 to 2013 in both the rounds.

Similar kind of studies been performed in different parts of the world to emphasize and analyze the importance of proficiency testing. One such extensive study performed in African region involved the distribution of PT panels to participating laboratories from 2002 to 2010, with a frequency of accurate detection, of anti-HIV-1 and/or anti-HIV-2 antibodies in the PT panels, ranging from 93% to 100%.[8] Accurate proficiency testing results are an integral part of laboratory accreditation process. The importance of proficiency testing procedure is shown by the fact that, to be recognized by WHO AFRO (Africa Regional Office), a laboratory must have scored, for each test, 80% or better on the two most recent PT panels.[9]

The analysis of total 8194 (4992 BB and 3202 ICTC) samples for quality control gave a platform for feedback on the performance of HIV testing in BBs and ICTCs, which can be of immense help in taking corrective actions in different factors affecting the quality of the tests performed. This has also provided an opportunity to find out discordant results that would otherwise have gone undetected.

Rapid tests with immediate test result are popular in the USA and Canada. Studies performed in these regions also emphasize on good laboratory quality control practices, QA, and participation in HIV proficiency testing to ensure accurate, timely, and clinically relevant laboratory results.[10]

German Medical Association has given immense stress in QA in their recent guidelines of diagnosis of infectious disease. These guidelines are mandatory for all the German laboratories and Section B2 of the guideline includes QA of qualitative analyses in laboratory diagnosis of infectious diseases including HIV.[11]

WHO guidelines for accuracy and reliability of HIV rapid testing pointed out some limitations of retesting and proficiency testing, which the laboratories should keep in mind while conducting retesting and proficiency testing. In case of retesting of specimens in many countries, there is lack of capacity at the NRL for retesting the large number of samples and for conducting the needed analysis of data. Long delays in completing the retesting results in delayed identification of problems. So, WHO has pointed the operational issues that need to be considered if a retesting program is to be implemented. Finally, statistical analysis shows that for low-volume sites, a very large percentage of samples would have to be retested to detect errors. In proficiency testing for HIV rapid tests, the panel of specimens sent to the testing site will not necessarily be tested by all staff, so it is not a good measure of individual performance. The sample size is small, so the ability to detect errors is impaired. Also, preparing and distributing specimens for proficiency testing may be cumbersome for NRLs. Proficiency testing is provided in some locations, and when available, it is a useful tool in combination with onsite monitoring.[12]

Conclusion

Established as an HIV testing center in 1992, SRL-CHT-LHMC has come a long way and obtained National Accreditation Board for Testing and Calibration Laboratories accreditation in 2013. During this phase, it has established a well-coordinated network through EQAS with its linked ICTCs, BB, and its mentor NRL [i.e., National Centre for Disease Control (NCDC), New Delhi]. Apart from conducting EQAS activities, SRL-LHMC is regularly conducting training programs for the technicians of the linked centers and help them to resolve any issues related to testing of HIV. Through all these activities, SRL-CHT-LHMC is providing constant support, encouragement, and opportunities for improvement of its linked centers.

A strong commitment from top-level managers is essential for the success of the overall quality program. This commitment is important at all levels, and national laboratory leaders will need to both provide strong leadership at the national

level and motivate and help laboratory managers throughout the country to understand the system and commit to its success.

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