Research Article

A retrospective study of quality management system at national reference laboratory in Mumbai - Highlighting quality in HIV testing

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ABSTRACT

Background: National reference laboratory (NRL) is a NABL certified center of HIV testing at National Institute of Immunohaematology. It has 2 union territories and 8 medical colleges as state reference laboratories (SRL's) with integrated counseling and testing centers (ICTC's) and blood banks (BB). It performs activities such as external quality assurance scheme, retesting of samples, and training programs. **Objective:** A retrospective study was carried out from 2012 to 2016 highlighting NRL's unceasing efforts in maintaining stringency in the quality management system. **Materials and Methods:** Verification and calibration of pipettes, evaluation of hands-on expertise of technicians for Combaids assay and ELISA testing were assessed. Retesting of samples as part of the quality check was performed. Panel proficiency testing of all the centers was done twice annually. **Results:** In 2013 and 2016 (81% and 36% pipettes) passed calibration, respectively. Evaluation of Combaids assay was done on the scale of excellent, medium, and poor. In ELISA testing, 53% followed standard operating procedure, 42% followed good laboratory practice, and 5% showed reproducibility in results. Evaluation of BB technicians was done using the borderline reactive sample in January 13, September 13, and January 14 showed correct results by 5%, 0%, and 11%, respectively, while 78% ICTC technicians gave correct results in Combaids assay. Proficiency testing showed few discordant results every year with need for calibration and technical assessment of personnel while total retesting samples tested from year 2012 to 2016 were 419. **Conclusion:** NRL emphasizes on the preparation of borderline sample as in-house quality control sample, calibration of equipments, training regarding same for assuring reliability of test results.

KEY WORDS: Borderline Sample; Calibration; Good Laboratory Practices; Standard Operating Procedure; External Quality Assurance; State Reference Laboratorie; National Reference Laboratory

INTRODUCTION

HIV/AIDS is a pandemic globally where approximately 70% of the final results are dependent on laboratory testing which forms an integral part for medical diagnosis.[1] National AIDS Control Organization (NACO) is functioning as a part of Indian Ministry of Health and Family Welfare since 1992. It governs all activities for prevention and control of HIV/AIDS in the country.[2]

NACO has developed four-tier structure to implement quality management system (QMS) including EQA program for HIV diagnosis at the national level. National AIDS Research Institute (NARI) at Pune forms the apex followed by national reference laboratories (NRLs). Then, underneath state reference laboratories (SRLs) are linked to integrated counseling and testing centers (ICTCs) and blood banks (BB) which are actually active testing sites. Further, NRLs conduct

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EQA workshop for linked SRLs with their District Reference Laboratories (DRLs) once in 6 months. NRLs assess SRLs with 8 sera panel and DRLs with 4 sera panel. SRLs and DRLs test the panel sera and report within 7 days of sample receipt. NRLs analyze the reports and accordingly send the performance report within 15 days. NRLs also send compiled report of respective SRLs and DRLs to NARI and NACO. Discordance at any center is notified necessitating further investigation and rectification.

Furthermore, routine quality checks or retesting is a part of external quality assurance scheme (EQAS) (to monitor routine work in HIV testing centers). On quarterly basis, NRLs receive 5% negative and 20% HIV positive samples of the total number tested by SRLs which, in turn, receive samples in the same proportion from their DRL's collection for the period from 1st to7th of January, April, July, and October. Hence, the authenticity and appropriate final results are analyzed through QA program, and any error in reporting is followed by investigation and prompt corrective action.

HIV testing is offered in about 6000 ICTCs under NACO using an algorithm of serial testing by three rapid assays with different HIV antigens or principles. The first assay is the screening test with the highest sensitivity and the other two rapid assays with highest specificity are procured by NACO. Hence, laboratories providing a diagnosis of HIV should be firmly committed to QA, continuous evaluation, and data collection procedure. Errors can potentially occur during many processes that take place from requisition of test until the release of the results. A proper quality management system is necessary for rectification of any error and maintenance of good quality of testing. QA includes quality control (QC) along with continuous quality improvement which together forms the fundamental components of QMS.

QA makes sure that the protocols of HIV testing are latest and equipment in use are well maintained. Whereas, QC as a part of QA ensures the validity of the test run, continuous education and competency assessment of staff members of the organization down the line are also prerequisites in maintaining a quality system.

Every stage of QA, namely pre-analytical, analytical, and post-analytical should be monitored meticulously by each HIV testing laboratory to attain the quality of international standard. As a part of the pre-analytical phase, calibration and maintenance of equipment along with an assessment of competency of the technician are of paramount importance. Calibration of an instrument is necessary for assurance of reliability and accuracy of results produced on its use. QC using both internal and external controls (EQC) and generation of results on proper interpretation comprises the analytical phase. EQAS monitors overall performance and identifies reliability and accuracy of HIV testing results obtained from all the participating laboratories evaluated at a given time. Furthermore, retesting and proficiency testing (PT) which is important in assessing technical competency and onsite technical conditions form the intrinsic part of HIV EQA.[3]

HIV testing center situated in transfusion-transmitted diseases department of national institute of immunohematology, (NIIH) ICMR in KEM hospital campus is one of 13 NRLs in the country. This NRL caters to four SRLs each from Mumbai and Madhya Pradesh. In addition, 7 peripheral centers from 2 union territories, i.e., Daman and Diu and Dadra Nagar Haveli are directly linked making a sum total of 319 HIV testing centers affiliated to it.

NRL, NIIH activities include preparation of panel and implementing proficiency testing (EQAS) biannually for SRLs along with their ICTCs and BBs, hands-on training of technicians, resolving indeterminate status, and/or discordance on samples referred from SRLs, testing of routine QC (RQC) samples, confirmation of HIV-1 and 2, and HIV sentinel surveillance as a part of QA program.[4] Furthermore, NRL performs in-house calibration of equipment such as micropipettes, freezers-refrigerators, and centrifuges using standard weight box, thermometer, and tachometer, respectively, as per national accreditation board for testing and calibration (NABL) guidelines.

NACO is making efforts for NABL accreditation for the strengthening of all HIV testing laboratories from apex to peripheral centers in the four-tier system, as per the norms of International Standard Organization.[5] It is challenging to maintain such quality, especially in resource-limiting settings, in spite of all the efforts at national level.[6] Accreditation scheme in India is voluntary, and unless appropriate wise steps are taken to encourage the laboratories for adopting quality practices, it may take very long time to fill the gaps in between.[7] NRL with its unremitting efforts operates within the principle of QA in providing quality HIV testing and encourages all HIV testing laboratories in the network underneath it for the same.

This is a retrospective study from the year 2012 to 2016 with an objective to show the journey of quality maintenance in NRL and its unceasing efforts to bring stringency in the quality system.

MATERIALS AND METHODS

In 2013 and 2016 all the laboratory technicians from Mumbai SRLs were instructed to get their micropipettes to NRL, NIIH for verification of calibration. In 2013, a total of 78 micropipettes were received while 62 micropipettes were received in 2016. Verification of calibration was done gravimetrically with a volume of 10 µl, and if the micropipette failed at this volume, it was verified at 100 µl as per the

procedure described by Project Concern International India (PCI). In 2016, a program was conducted by Share India at NRL to train technicians from linked centers in Mumbai for in-house calibration of equipment.

Combaids (Span diagnostics) was the 1st rapid assay where the strips are coated with immobilized antigens, and colored reaction after formation of AB-Ag complex easily detects HIV infection. The laboratory technicians from ICTCs and BBs were assessed for their hands-on expertise, good laboratory practices (GLPs), and interpretation skills for reporting. Laboratory technicians from ICTCs (n = 63) and mobile van ICTCs (n = 3) were given EQC (External QC - low positive control) for the first rapid assay. Laboratory technicians from BBs (n = 19) had to test EQC by ELISA (SD).

EQC was appropriately prepared by NRL, NIIH by selecting a precise dilution of a known HIV positive sample with a negative sample for a particular assay as per NACO guidelines. The same pair of HIV positive and negative sample stored at −80°C was used for making EQCs specific for three rapid assays, ELISA and western blot assays simultaneously. For stringent QC, EQCs were run in duplicates through intra and inter ELISA runs followed by plotting Levy Jennings' chart to know if they were in the accepted range.[1] During technical training, EQC for the rapid assay (Combaids) had a standard result of +1.0 CI whereas, that for ELISA had E ratio in the range 1.06–2.15 (±2 SD).

In January 2012, NRL received 120 samples from all 8 SRLs and also, directly from DRLs under Union Territories of Dadra Nagar Haveli and Daman and Diu for retesting. Then, from 2nd quarter onward retesting was discontinued at NRL level from all SRLs. 14 samples were received only from Dadra Nagar Haveli while no sample was received for 3rd and 4th quarter. In 2013 (91 samples), in 2014 (85 samples), in 2015 (54 samples), and in 2016 (55) samples were received from both the union territories.

RESULTS

Verification of micropipette calibration was done gravimetrically in 2013. It was observed that 81% of the micropipettes passed micropipette calibration and the others failed as shown in Figure 1. Competency of ICTC technicians was checked on a rapid assay, namely combaids using different parameters such as pipetting technique, time of opening testing devices (antigen coated comb), dispensing of sample, checking of antigen coated combs, GLP, and rocking of combs while incubating and washing steps, angle of reading result on comb, and blotting of combs on a scale of excellent, medium and poor as shown in Figure 2.

BB technicians performed SD ELISA as per NACO strategy. Of them, 53% followed the standard operating protocol

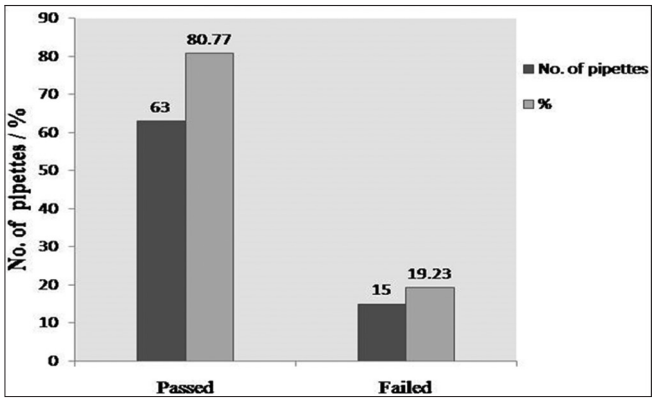

Figure 1: Verification of calibration of micropipette

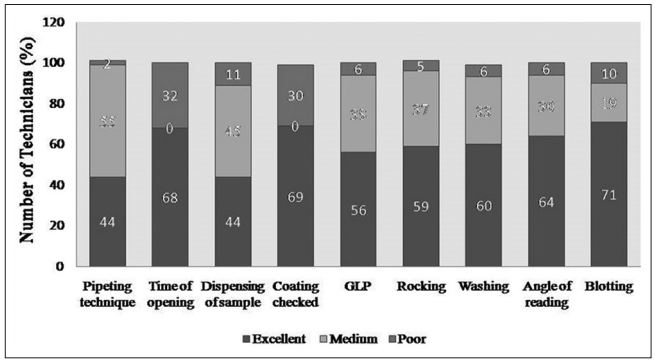

Figure 2: Evaluation of Combaids testing assay

standard operating procedure properly, 42% showed GLPs and, only 5% showed reproducibility in the results as shown in Figure 3. They were given borderline HIV positive sample of whom only 5%, 0%, and 11% showed proper results in January 2013, September 2013, and January 2014, respectively. On the contrary, 78% of ICTC technicians showed accurate results for borderline HIV positive sample by Combaids as shown in Figure 4.

In 2016, calibration status of pipettes from ICTCs was checked at NRL. In this 30%, 23%, 43%, and 44% of pipettes were in-calibration for linked centers to four respective SRLs from Mumbai as given in Figure 5.

From 2012 to 2016, retesting of 419 samples showed concordant results. Proficiency testing of all affiliated SRLs had 100% concordance except one from Madhya Pradesh showed 87.5%, and the other one from the Union territory of Dadra Nagar Haveli had only 25% concordance in 2014. In case of centers linked to SRLs 3% (total 261 centers) in 2012, 3% (336) in 2013, 7% (255) in 2014, 2% (222) in 2015, and 3% (318) in 2016 gave discordant results.

DISCUSSION

NRL highlighted the need for calibration and importance of borderline reactive sample to detect minor errors while performing HIV testing. Further, distribution of panel sera

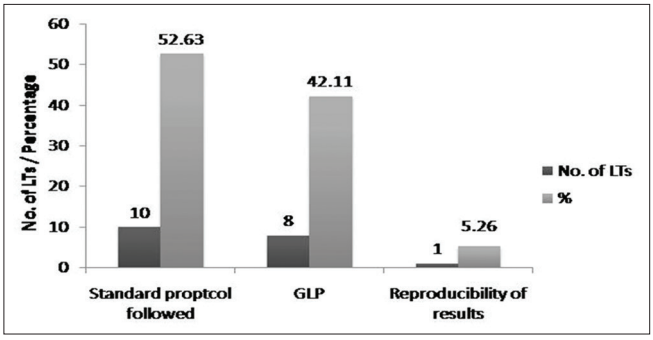

Figure 3: Technical evaluation of blood bank technicians

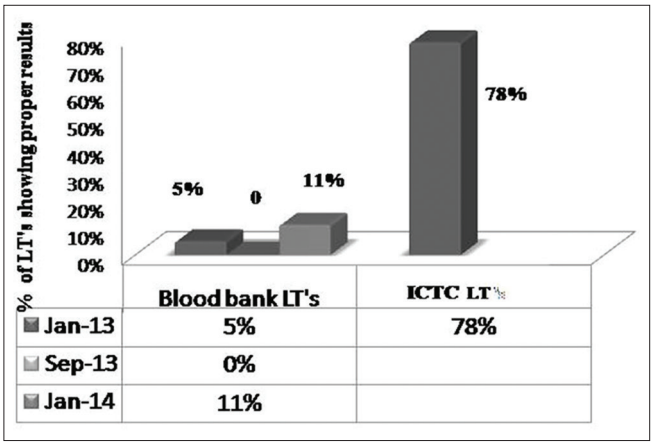

Figure 4: Evaluation of technicians (LT's) using borderline reactive sample

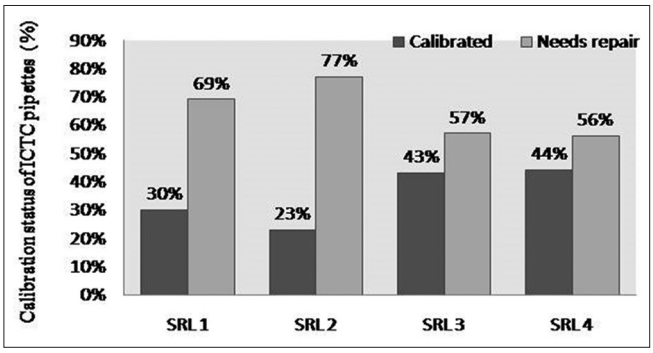

Figure 5: Pipette calibration of integrated counseling and testing centers under Mumbai state reference laboratory

biannually and retesting quality check samples which form a part of the external quality assessment scheme (EQAS) helped to assess the performance of the linked centers. Investigations necessitated for the observed discordance during EQAS revealed need for equipment maintenance and training of personnel.

A stepwise approach by studying the key elements of accreditation leads to a strengthening of a laboratory. Quality cannot be achieved by accident but is a result of good planning. NRL follows NACO guidelines for QA program. Moreover, the department progressed in leaps and bounds post accreditation.

In low resource settings, the preliminary testing by ELISA is substituted by rapid tests (RTs) to suffice increasing requirement of HIV testing.[5] As per ongoing NACO algorithm, 3 RTs are being used to give final labeling as HIV positive. These assays are based on different principles such as dot immunoassay, immunochromatography, and immunoconcentration giving final results in the form of colored dot, line, and/or agglutination, respectively. The first RT giving non-reactive result on the sample is reported as negative. Thus, the 1st RT contributes significantly to final reporting of the results. BBs adopt ELISA whereas the majority of ICTCs face a challenge of maintaining and ensuring the quality of HIV testing with the expansion of rapid assays.

Usually, for QC, internal controls available in both rapid and ELISA kits are artificially prepared for known substances. However, the external control sample especially borderline reactive sample may be developed in-house by mixing positive and negative serum samples. On validation, it may be included in all rapid assays during HIV testing. Such stringent control when running along with patient samples helps to rectify/nullify any error in the final report, and thus forms the basic component of QC procedure.[8] Inclusion of such borderline reactive control is important in detecting minor errors with OD values near cutoff in ELISA and interpretation of final results in the rapid assay. Hence, mistakes while evaluating unknown samples due to such variations can be easily curtailed.

At NRL, borderline HIV positive sample is used as a part of QC so as to minimize errors in HIV testing. Such hands-on evaluation not only underlines the significance of borderline sample in mitigating mistakes resulting from poor performance but also eliminates erroneous interpretation in reporting of blinded samples which may otherwise go undetermined. HIV RT is simple and easy to perform giving precise results if performed as per kit protocol. Still mistakes can occur at any point of testing due to varied potential reasons. The fundamental challenge faced by increasing use of rapid assays is reporting of final results as faint and weak lines due to interpretation error by technicians. Such mistake may undergo unnoticed leading to a considerable level of false positive reporting.[9] Preferably rereading of final results of the assay by the second reader is done for rapid diagnostic tests with repetition of testing with the new device and fresh sample in case of disagreement has also been reported.[10] Hence, it is extremely important to use both internal controls (negative and positive) along with external control/s while running HIV rapid assays to bring stringency in QC. While handling large number of testing samples, even a small error can lead to incorrect diagnosis which will further not only affect an individual but also the overall health of society with negative repercussions.[11]

A mandatory policy is usually established by the ministry of health with standard operating protocols mentioning the

frequency of using external QC samples. Usage of external QC is dependent on varied factors, one of them being condition of the kits in use. Environment factors and transportation conditions can at times go out of control finally affecting the quality of kits. This is a constant challenge warranting kit validation frequently. QA in HIV testing is mentioned in several reports but the inclusion of borderline positive sample in both Rapid and ELISA assays is still unassured.

Being an accredited lab, NRL regularly executes in-house calibration of several instruments along with micropipettes. Accordingly, calibration process for few linked centers was performed showing contemporaneously malfunctioning of micropipettes used for testing. Calibration of instrument for delivering precise sample volume is important. Not only defective micropipettes and mechanical liquid handlers lead to poor dispensing of sample volume but also incompetency in handling pipettes can influence laboratory results.[12] Through the years there is a substantial development in analytical testing by laboratories due to technological up-gradation and marked improvement in a standardized protocol. Still, notable errors are highlighted at different analytical phases of the testing. One of the Clinical Institute report released in 2004 has revealed how calibration error during practicing guidelines influence clinical decision of number of patients. Hence, understanding calibration and traceability of standard instruments in use by the technical personnel is crucial. Although calibration of equipments is usually performed by external agencies, in-house calibration of some instruments can enhance reliability and thereby testing accuracy.[13] Further, preventive and corrective maintenance of all the equipment used in HIV testing through proper implementation of inventory is also of paramount importance.[14]

QC for HIV testing is implemented through a chain of SRLs, NRLs, and Apex laboratory. NACO has successfully established EQAS in each HIV testing center at district level through SRL which, in turn, linked to NRL. Such EQA challenges the laboratories' internal QC program and thus speculating effective testing performance.[15] Moreover, increasing number of HIV diagnostic laboratories throughout the country has practically included this quality check program for analytical advancement.[16] Due to low resource settings, not all laboratories under NRL are accredited, thereby making their participation in EQA very critical. Such evaluation not only increases the quality of a laboratory but also there is an improvement in customer satisfaction. Participation in EQA scheme along with internal QC is an intrinsic part of many laboratories.[17] Such implementation highlights the quality of HIV testing as well as the competency of technical personnel and hence, requisite training if there is obvious lack of handling skills. Although assays and policies used for HIV testing may differ with country conditions, it is a prime concern of different health ministries to maintain a coherent and robust quality management system (QMS).[18] Hence, maintaining principal requirements of HIV testing as per international laboratory standards will strengthen the QMS of every testing center in resource-limiting settings.

World Health Organization established in United Nations is an important agency involved in international health policy and standards. It has currently suggested that many health-related settings worldwide give inadequate attention to the QA and incorrect diagnosis is observed due to varied intrinsic parameters and methods. With increasing focus on client satisfaction and their demand, assurance in analytical phase is enhanced over time. However, the extreme probability of errors in pre- (before the test) and post-examination (after the test) is also reported and required to be limited so as to maintain the total quality of laboratory settings.[19] Hence, regular participation in external assessment program and compliance with the laboratory standard protocol will not only upgrade working productivity but also decrease the rate of errors substantially thus improving client service. In developing countries, maintaining standard of testing is difficult due to the scarcity of resources leading to underrated laboratory performance. Accordingly, various health organizations worldwide have publicized in laboratory quality system conferences about a need of stepwise approach. National laboratories with advanced infrastructure are fulfilling internationally accepted accreditation standards, but the laboratories with limited resources should make efforts strategically to attain least requirements of such standard.[20] Such global challenge to strengthen the lab system and cope up with the ever-increasing demand for improvement in testing services has been addressed by implementation of certain approaches especially in resource-limited countries to resolve the oversight.[21] While proper and timely funding can help to develop the strategic plan, there are few countries that have started with Harmonization of HIV testing services and standardization of procedures. Although such establishment has shown mixed success, its progress and compliance in other areas globally need proper assessment.[22,23]

The strength of the study was emphasis given to stringency of QC which affects test results and hence reporting. Limitation of the study was an assessment of technicians from Mumbai only and not from other linked centers from Madhya Pradesh and two Union territories. Furthermore, the verification of calibration of pipettes was done only for linked centers in Mumbai.

CONCLUSION

To summarize on the insights of this study, there is a need of training with respect to equipment handling, correct execution of testing procedure, and inclusion of right QC sample/s. It is of utmost importance to include an external borderline positive control to achieve the highest standard of analytical quality for HIV testing at any given laboratory. Furthermore, it is necessary to maintain equipments properly and provision of training required for in-house calibration

especially of automatic pipettes for assuring reliability of test results.

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