Research Article

Improvement of laboratory testing services by implementation of quality assurance scheme

A Singh[1], R Sachan[2], S Kumar[2]

Sri Lankan Journal of Infectious Diseases 2024 Vol. 14(1): E52:1-9

DOI: https://doi.org/10.4038/sljid.v14i1.8541

Abstract

Introduction: The National AIDS Control Organization (NACO), Government of India and the Uttar Pradesh State AIDS Control Society (UPSACS) established the State Referral Laboratory (SRL) in the Department of Microbiology, Motilal Nehru Medical College, Prayagraj for HIV testing and monitoring of linked HIV Counselling and Testing Services (HCTS) in Uttar Pradesh.

Methods: This is a retrospective study conducted from 2008 to 2021. In this evaluation period 10144 serum samples (9580 HIV negative, 555 HIV positive and 9 discordant) were received for retesting at SRL. The Quality Control serum samples received from testing sites consisted of 5% negative and 20% positive from all tested samples collected in the first seven days in the External Quality Assurance Scheme (EQAS) quarter month i.e. January, April, July and October. All samples were tested using rapid diagnostic kits procured by NACO/UPSACS with NACO testing strategy. All linked centres with SRL received 4 coded serum samples for proficiency testing (PT) which were tested similarly**.**

Results: Of 10144 serum samples, 99.92% reported correct results and 0.08% reported discrepancy due to various issues. Similarly in the proficiency testing programme, of 4072 samples tested between 2010 to 2021 by testing sites, 4061 (99.07%) reported concordant results and the remaining14 (0.34%) samples reported indeterminate results. Of the 4072 samples, 14 shows discordant results by three different rapid tests based on different principles. Hands on training were provided for technicians who reported indeterminate results in PT or retesting.

[1]*Department of Pulmonary Medicine, Motilal Nehru Medical College, Prayagraj, 2l1001, U.P. India.*
[2]*Department of Microbiology, Motilal Nehru Medical College, Prayagraj, 2l1001, U.P. India.*

Address for correspondence: Dr. Reena Sachan, Department of Microbiology, Motilal Nehru Medical College, Prayagraj, 2l1001, U.P India. Email:drreenal26@gmail.com; Telephone: 9721410660;
https://orcid.org/0000-0002-1606-9585

Received 12 December 2022 and revised version accepted 25 October 2023. Published on 5 February 2024

Conclusion: The tremendous increase in quality testing at peripheral centrally linked centres increase the reliability of HIV diagnosis at ground level. The study also concluded that continuous monitoring and mentoring of technicians of the diagnostic laboratories under EQAS improves their quality for HIV diagnosis.

Keywords: *HIV, NACO, EQAS, PT, RT*

Introduction

India has the third highest number of probable People Living with Human Immuno-deficiency Virus (PLHIV) in the world. According to 2019 estimations, the number of PLHIV cases in India was 23.19 lakhs and the maximum percentage were in the 15-49-year age group. The highest numbers of PLHIV were reported in Maharashtra, Andhra Pradesh, Karnataka, followed by Uttar Pradesh and other states of India.[1]

The first phase of the National AIDS Control Programme (NACP-I) was launched by the Government of India in 1992 to combat HIV infection and Acquired Immuno-Deficiency Syndrome (AIDS) in the initial stage itself. However, with the evolving trends of the HIV/AIDS epidemic world-wide, the focus of subsequent phases of the programme (NACP-II in 1999, NACP-III in 2007 and NACP-IV in 2012) shifted from raising HIV/AIDS awareness to behaviour change, from a national response to a more decentralised response and to increasing involvement of various NGOs and networks of People Living with HIV (PLHIV).[2]

After successful completion of the National AIDS Control Programmes I, II, III and NACP IV, India has demonstrated 37% reduction in the annual new HIV infections since 2010 and more than 86% since 1997.

Due to anti-retroviral therapy (ART), the annual AIDS deaths have shown a steady decline since roll out of the free programme in India in 2004; it is estimated that around 1.5 lakh lives have been saved due to ART up to 2011. Children less than 15 years accounted for 7% (1.45 lakhs) of all infections in 2011. Of all HIV infections, 39% (8.16 lakhs) were among women.[3]

NACO has implemented the External Quality Assurance Scheme (EQAS) under Phase III (2007-2012) to provide qualitative enhancement in HIV Counselling and Testing services (HCTS) in continuation with NACP-IV Phase.

EQAS includes three vital components:

1. Retesting
2. Proficiency Testing (PT)
3. On site monitoring of testing sites

A key programme of EQAS is retesting, where samples already analysed at other HIV testing laboratories are retested at reference laboratories, which enables inter-laboratory (*Testing Sites to Referral laboratories*) comparison.[4] HIV antibody detection in serum/blood samples possesses several technical parameters which require retesting at specific intervals to determine the quality of a particular laboratory and reliability of results. Quality checks of HIV antibody testing by a rapid method is necessary for testing sites/laboratories because tests are performed outside a traditional laboratory and kits are single-use and cannot be subjected to the usual quality control methods used by standard laboratories.[4] Retesting is the process by which results of a particular laboratory are counterchecked by a reference laboratory to ensure the quality of performance at testing sites.

The second activity of EQAS is "Proficiency Testing" (PT) that refers to the programme in which multiple blindfold samples are periodically sent to groups of laboratories for analysis and/or identification; the results of each laboratory are compared with the true status of the samples. It is very necessary that PT panel samples are run along with daily routine samples.

Quality Assurance (QA) is the planned sequence of procedures that ensure that a correct result is achieved in a standard, reproducible and traceable manner and mistakes are found and corrected to avoid adverse outcomes.[4] It is essential to achieve good quality results, and the staff of the laboratory should be aware of procedures for quality performance. To increase the competency of every staff member, continuous education and training is a necessity.

The State Reference Laboratory (SRL), Department of Microbiology, Motilal Nehru Medical College, Prayagraj supervises and mentors 54 HIV Counselling and Testing Services (HCTS) under the guidance of the National AIDS Control Organization (NACO) and Uttar Pradesh State AIDS Control Society (UPSACS).The SRL implements quality control (QC) testing, proficiency panel testing (PT), training programmes and onsite monitoring programmes under EQAS activity.

In 1999, the Integrated Counseling and Testing Center (ICTC) was established in the Department of Microbiology, Motilal Nehru Medical College (Government Medical College), Prayagraj. In 2007, under NACP-III, NACO established the State Reference Laboratory (SRL) in the Department of Microbiology to strengthen laboratory services at field level. In the early years of EQAS activity, only a small number of laboratories participated in retesting programmes under EQAS. Stability of HIV diagnostic laboratories at community health centres and district hospitals did not meet the required standards as HIV testing was done along with other tests in general pathology. There was no separate laboratory for HIV diagnosis.

After establishment of the SRL in NACP-III, regular EQAS workshops were conducted biannually for linked HIV Counselling and Testing services (HCTS) and coordination with the National Reference Laboratory (NRL) was established under the guidance of UPSACS to improve HIV testing quality standards at the field level. The SRL closely monitored QC testing, proficiency panel testing (PT), training programmes and onsite monitoring of the

linked testing sites to enhance the quality of HIV testing at peripheral centres so that none of the testing facilities reported false positive or false negative results. Such monitoring also ensured that all HIV positive persons were enrolled at their respective ART centres for further basic testing, diagnosis, and treatment.

Aims and Objectives

This retrospective study was carried out to determine the performance of activities done under EQAS which included NRL, SRL and linked Integrated Counseling and Testing Centers, and supervision and mentoring of the laboratory personnel involved in HIV testing. Following were the objectives of this study:

1. Determine the effectiveness of referral laboratories at field level and coordination between NRL and testing sites.
2. Check the functionality and improvement of testing sites after training and hands-on workshops.
3. Identify the discrepancies in test results from different laboratories.

Methods

The NACO established SRL in the Department of Microbiology to improve the quality of HIV testing at linked testing sites and ensure 100% participation in the EQAS programme along with continuous mentoring and monitoring of sites. SRL Prayagraj monitors and supervises 54 testing sites, and each testing site collects and stores samples in the first week of each quarter and sends 5% of HIV negative serum samples and 20% of positive serum samples from them to SRL for retesting. These sites also receive proficiency panels from the referral laboratory.

Inclusion criteria of retesting: Samples were collected at testing sites in the 1st week of each quarter (*i.e. January, April, July, and October*) and 5% of negative and 20% of the positive serum samples were sent to SRL, with triple layer cool packing by specified trained personnel, as per transport guidelines of WHO/NACO, in the 2nd week for retesting. Samples received at referral laboratories were tested in accordance with NACO strategy by using HIV diagnostic rapid tests based on different principles such as immuno-chromatography, immuno-concentration, and immuno-blot assays which were procured by UPSACS/NACO as per availability.

As per NACO's strategy:

1. The first rapid test kit should have high sensitivity and the next two rapid test kits should have high specificity.
2. The three kits should be of three different principles and have different antigen preparations.[2]

Exclusion criteria for retesting: Samples not received within the 14th of the EQAS month were liable to be rejected for this activity. Serum samples which satisfied the inclusion criteria were processed as per protocol and reports sent back to the respective HCTS.

In the EQAS retesting programme, the SRL, Department of Microbiology, received 10,144 samples between 2008 and 2021 from linked HCTS for quality control checking. All samples were tested as per NACO protocol and reported back to linked respective centres within the turnaround time (TAT) i.e.*7 days*.

In 2008, 2009 and the first quarter of 2010, participation of laboratories in retesting activity was low, and limited numbers of samples were received by the referral laboratory. Participation of ICTCs increased in subsequent quarters of the year from April 2010 and reached up to 98-100%, after close monitoring by SRL under EQAS activity.

Proficiency Testing: For the PT programme, serum/plasma samples were received from the National Reference Laboratory (NRL i.e. National Institute of Biologicals, Noida) annually in 2008, and biannually from 2010. From 2010, SRL received PT samples from NRL for itself as well as for linked peripheral centres. All the PT samples for SRL were tested as per protocol and reports sent back to NRL within TAT. PT panel samples received from NRL for linked testing sites were aliquoted and provided to peripheral centres in triple layer packaging system for testing within a specified time.

Results

Retesting at SRL was done on 10,144 samples received from associated linked centres. During the study period, 9580 (94.44%) serum samples were reported as negative for HIV antibodies, 555 (5.47%) reported as positive for HIV antibodies, and the remaining 9 (0.08%) reported as discordant HIV positive in 2008 and 2009. **(Table 1 and Figure 1)**

Table 1: EQAS retesting results of testing sites (i.e. ICTCs and Blood Banks)

Year	***Total number of samples received***		***Total number of negative samples received***		***Total number of positive samples received***		***Indeterminate /Discordant***	***% Positivity***
	n	***% of total no.***	***n (% of annual no.)***	***% of total no.***	***n (% of annual no.)***	***% of total no.***		
2008	314 (100)	3.09	280 (89.1)	2.92	28 (8.9)	5.04	06 (1.9)	8.92
2009	361 (100)	3.56	331 (91.7)	3.45	28 (7.7)	5.40	01 (0.5)	7.76
2010	509 (100)	5.01	476 (91.7)	4.96	32 (8.1)	5.76	01 (0.2)	6.28
2011	586 (100)	5.78	526 (89.6)	5.49	60 (10.4)	10.81	00 (0.0)	10.24
2012	756 (100)	7.45	692 (91.5)	7.22	64 (8.7)	11.53	00 (0.0)	8.47
2013	534 (100)	5.26	510 (95.5)	5.32	24 (4.5)	4.32	00 (0.0)	4.49
2014	894 (100)	8.81	856 (95.7)	8.93	38 (4.3)	6.84	00 (0.0)	4.25
2015	847 (100)	8.34	799 (94.3)	8.34	48 (5.7)	8.64	00 (0.0)	5.67
2016	931 (100)	9.17	890 (95.5)	9.29	41 (4.5)	7.38	00 (0.0)	4.40
2017	742 (100)	7.31	692 (93.2)	7.22	50 (6.8)	9.00	00 (0.0)	6.74
2018	845 (100)	8.33	793 (93.8)	8.27	52 (6.2)	9.36	00 (0.0)	5.82
2019	1185 (100)	11.68	1130 (95.3)	11.79	55 (4.7)	9.90	00 (0.0)	4.64
2020	661 (100)	6.51	642 (97.1)	6.70	19 (2.9)	3.42	00 (0.0)	2.87
2021	979 (100)	9.65	963 (95.7)	10.05	16 (4.3)	2.88	00 (0.0)	1.63
	10144		**9580**		**555**		**9**	

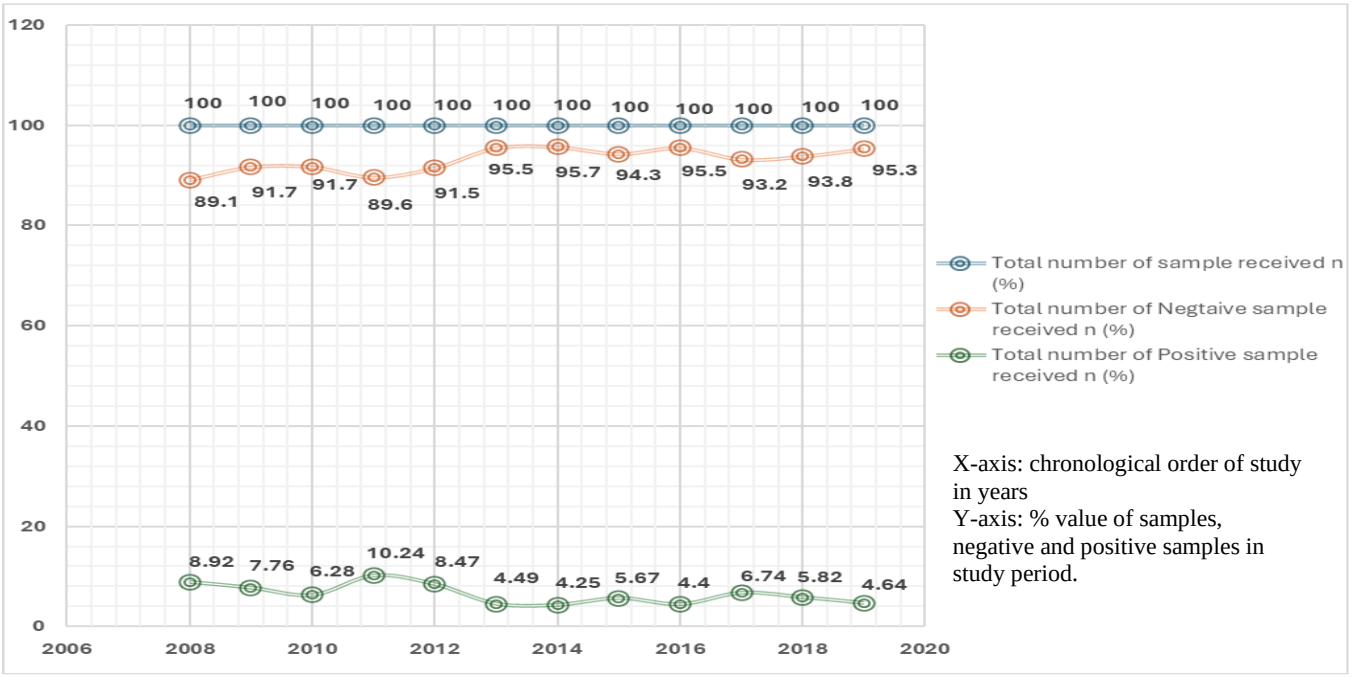

FIGURE 1: EQAS retesting from testing site to referral lab

Discrepancies were found in 2008 and 2009 with no discrepancies found in subsequent years.

SRL also received the proficiency panel testing from NRL and achieved 100% score in the proficiency testing programme conducted by NACO through NRL except in one PT panel programme in 2011**(Table 2)**.

Table 2: Status of Proficiency Testing (i.e. referral lab to testing site)

Year	***Total number and % of samples provided as PT***		***Total number and % of concordant results***		***Total number and % of discordant results***	
	n	***%***	***n***	***%***	***n***	***%***
2010	164	4.02	164	100	0	0
2011	192	4.71	188	97.91	4	2.08
2012	376	9.23	373	98.67	3	0.79
2013	440	10.80	440	100	0	0
2014	440	10.80	440	100	0	0
2015	184	4.51	184	100	0	0
2016	208	5.10	205	98.55	3	1.44
2017	416	10.21	416	100	0	0
2018	412	10.11	410	99.51	2	0.48
2019	408	10.01	408	100	0	0
2020	416	10.21	416	100	0	0
2021	416	10.21	414	99.51	2	0.48
	4072		**4061**		**14**	

Discussion

In India, the EQAS programme was implemented by NACO under NACP-III activity. It functions with 1 Apex Laboratory, 13 NRLs and 117 SRLs in government medical colleges, hospitals, and thousands of HIV testing sites all over the country, which includes medical colleges, district male/female hospitals, community health centres and primary health care units.

The EQAS programme is linked with UPSACS for effective functioning under NACO.[5]

Participation of laboratories in the retesting programme was low in 2008 and 2009 as it was the initial stage of the programme, and there was lack of monitoring and awareness of the programme. The number of samples tested during that period was therefore too low and some samples gave discordant results as well.

After 2010, the EQAS programme was strongly recommended by NACO, and participation of laboratories became mandatory. NACO officials started proper monitoring and supervision of linked centres at each step. Due to this, participation in EQAS retesting and proficiency testing increased tremendously and reached up to 98-100%. The discordant percentage dropped to zero due to continuous training at refresher training/induction training and biannual hands-on workshops.

The State Reference Laboratory is involved in quality control testing (*i.e. retesting*), proficiency panel testing, organising various EQAS workshops, and induction and refresher training to improve HIV testing at linked testing sites in coordination with NACO and UPSACS. SRL provides hands-on training to testing sites for this activity. Financial aid is provided by NACO through UPSACS. SRL ensures participation of centres in QA programmes which are followed by corrective actions.

SRL conducts training and hands-on workshops biannually, and technicians at linked centres participate in this activity. In training programmes/workshops, SRL technical resource persons instruct technicians on how to adhere to the Standard Operating Procedures (SOPs) in the use of correct pipetting technique, use of new tips for each sample, handling, maintenance, regular monitoring and decontamination of equipment, biomedical waste management, prevention of sample contamination, and about transcriptional errors during reporting. Pre- and post-training questionnaires were used during workshops to assess the effectiveness of training and find out the overall gain to participants.[6]

The main and most important component of the QA programme is the SOP. All laboratory personnel must be familiar with the procedures of every laboratory activity. The SOPs are reviewed and authorised every year with no deviations from the procedures in order to get correct results. All aspects of sample handling, right from arrival to reporting must be monitored, documented, and subjected to quality control procedures.[7]

Along with SOPs, chemical reagents and equipment performance must be monitored periodically to detect any changes/deviation in quality and integrity of results. Storage of diagnostic kits and their reagents also plays an important role in quality and reproducible results.[8] If negative and positive control lines or dots on rapid test devices/cassettes are not properly visible, it will lead to misinterpretation of results. Calibrating of pipettes and centrifuges annually should be done in the laboratory. The temperatures of the refrigerators are monitored by a digital thermometer where HIV testing kits are stored. Expired kits should not be used.

The QA programme should be in place to continuously assess and improve the performance of laboratory results which is important for physicians in guiding the patient for treatment and further management.[9] It is also suggested that regular audits of laboratory procedures and reviewing incident reports should be carried out by the senior staff who are in charge and should be discussed with the Director or Head of the Laboratory**.**

Conclusion

EQAS is an early word of warning scheme that 'identifies areas which require immediate attention' and includes training of laboratory personnel.[10] It also benchmarks performance against other laboratories. As a participating laboratory, SRL is trying to improve our laboratory/testing sites practices by regular quality control of test kits, procedures, and equipment.[11] During this period (2008-2021), HDL-SRL improved their coordination network through EQAS with its linked testing sites and NRL (National Institute of Biologicals, Noida). Through all these activities, SRL is providing constant support, encouragement, and opportunities for improvement of its linked centres. Success of the EQAS programme is based on every tier of laboratory, their staff and management.

The percentage of false positive and false negative results decreased tremendously with time after successful running of the EQAS programme as well as monitoring and mentoring of the testing sites and staff. Rejection of samples also decreased due to proper training on labelling, transportation, storage, and handling. None of the testing sites now give discordant results in the retesting programme. During biannual workshops, pre- and post-training questionnaires show that technicians benefit from hands-on experience, and it increases their efficiency for quality control procedures. Regular monitoring along with training and competency evaluation of staff is the key to success in any programme. The External Quality Assurance Scheme has significantly improved quality of testing at the testing sites.[12]

Declarations

Acknowledgement: Authors are thankful to Dr V. K. Gupta, Additional Director General Health Services, Principal Consultant, HAG Member, Quality Council of India for continuous support to maintain the quality services of SRL. Authors are also thankful to technical staff of testing sites, which collects the sample from patients prior to their verbal consent and sends samples to SRL for retesting. SRL Lab Technician Mr. Akhilesh Pandey has managed all responsibilities of testing and reporting.

Conflicts of Interest: The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

Funding: Not Applicable

Ethics statement: Ethical approval is not applicable for this article at our end, while informed/verbal consent has been taken by supporting staff at testing sites. Publication consent has been taken from heads of departments, and under their supervision and guidance, research has been carried out.

Authors' contributions:

Concept of review	: Dr. Reena Sachan, Dr. Sanjay Kumar
Data Selection and analysis	: Dr. Abhishek Singh, Dr. Sanjay Kumar
Methodology	: Dr. Reena Sachan, Dr. Sanjay Kumar
Supervision along with Validation	: Dr. Reena Sachan, Dr. Abhishek Singh
Writing and drafting	: Dr. Reena Sachan, Dr. Sanjay Kumar
Review& editing	: Dr. Reena Sachan, Dr. Abhishek Singh, Dr. Sanjay Kumar

References

1. India HIV Estimates 2020 Technical Brief, National AIDS Control Organization (NACO), New Delhi
2. National AIDS Control Organization (NACO) *https://naco.gov.in/nacp-iv-components*
3. Technical Report, India HIV Estimation 2012 National AIDS Control Organization (NACO)
4. Annual Report 2013-14, Department for AIDS Control, Ministry of Health & Family Welfare, Govt. of India.
5. Sherwal BL, Gogoi S, Gupta P *et al*. 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. *Int J Med Sci Public Health* 2015; 4:763-768. *doi: http://dx.doi.org/10.5455/ijmsph.2015.19122014154*
6. Sandle LN. The management of external quality assurance *Clin Pathol* 2005; 58:*141–144.* *doi: https://doi.org/10.1136/jcp.2003.015545*
7. Kabra S, Kanungo R. Monitoring quality of HIV testing at point of care facilities in India. *Indian J Med Microbiol*2012;30:129-30. *doi: http://dx.doi.org/10.4103/0255-0857.96635*
8. Sushi KM, Gopal T, Jacob SM, *et al*. External quality assurance scheme in a national reference laboratory for HIV testing in South India *World Journal of AIDS* 2012; 2:222-225 *doi: http://dx.doi.org/10.4236/wja.2012.23028*
9. Chaudhary R, Das SS, Ojha S, *et al*. The external quality assessment scheme: five years' experience as a participating laboratory. *Asian J Transfus Sci* 2010; 4(1):28-30. *doi:http://dx.doi.org/10.4103/0973-6247.59388*
10. Louis FJ, Excellent ML, Anselme R, *et al*. External quality assessment for HIV rapid tests: challenges and opportunities in Haiti. *BMJ Glob Health* 2018; 3(6):001074. *doi: http://dx.doi.org/10.1136/bmjgh-2018-001074*
11. Yerram S, Sripad DV, Prabod VS, *et al*. External quality assurance scheme (EQAS): Criteria for evaluating performance of a laboratory. *IOSR Journal of Biotechnology and Biochemistry 2018;* 4(4):16-20. *doi: http://dx.doi.org/10.9790/264X-0404011620*
12. Wattal C, Oberoi JK, Goel N, *et al*. Experience of Indian Association of Medical Microbiology External Quality Assurance Scheme Centre, New Delhi: Challenges and Quality Assessment of Clinical Microbiology Laboratories. *Indian Journal of Medical Microbiology* 2019; 37(2):163-172 *doi: http://dx.doi.org/10.4103/ijmm.IJMM_19_356*