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Proficiency Testing and Onsite Assessment of HIV Rapid Testing sites at Health Facilities enrolled in HIV Rapid Test Quality Improvement Initiative (RTQII) in Ethiopia

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This is to certify that the thesis prepared by Dereje Yenealem, entitled: **Proficiency Testing and Onsite Assessment of HIV Rapid Testing sites at Health Facilities enrolled in HIV Rapid Test Quality Improvement Initiative (RTQII) in Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations/Acronyms

AIDS	Acquired Immunodeficiency Syndrome
CDC	Centers for Disease Control and Prevention
DBS	Dried Blood Spots
DTS	Dry Tube Sample
EID	Early Infant Diagnosis
ENBB	Ethiopian National Blood Bank
EPHI	Ethiopian Public Health Institute
EQAS	External Quality Assessment Scheme
HEWs	Health Extension Workers
HFs	Health Facilities
HIV	Human Immunodeficiency Virus
HTCs	HIV Testing and Counseling Centers
HTS	HIV Testing Sites
NHTA	National HIV Testing Algorithm
PEPFAR	President's Emergency Plan for AIDSRelief
PICT	Provider- Initiated Counseling and Testing
PMTCT	Prevention of Mother-to-Child Transmission
POC	Point-of-care
PT	Proficiency Testing
RDTs	Rapid Diagnostic Tests
RTQII	Rapid Test Quality Improvement Initiative
SNNPR	Southern Nations Nationalities and Peoples Region
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
UNAIDS	United Nations Programme on HIV/AIDS
VCT	Voluntary HIV Counseling and Testing
WHO	World Health Organization

Abstract

Background: HIV rapid testing services must ensure that appropriate quality assurance programs and well understood testing algorithms are implemeted to obtain accurate and reliable test results. Proficiency testing programs are important for improving HIV rapid testing quality while conducting an onsite evaluation provides a chance to observe the overall testing situation and support on identified gaps.

Objective: To assess the performance of HIV Rapid testing sites in Rapid Test Quality Improvement Initiative(RTQII) enrolled health facilities through proficiency testing and onsite evaluation.

Method: Health facility based cross sectional study was conducted from August to December 2019on 159 HIV testing sites (HTS) in 41 Health facilities (HFs) in five administrative regions and two city administrations in Ethiopia. Characterized HIV dried tube specimen (DTS) PT panels were prepared and verified at the Ethiopian Public Health Institute (EPHI) and distributed to the sites. Also onsite evaluation, accompanied by checklists with structured interviews, was conducted. The collected data was analyzed by SPSS version 23 and chi square test was applied to identify the association between acceptable performance and different factors.Testing sites whose PT scores matched 100% with the expected result together with adherence to the National HIV Testing Algorithm(NHTA) were considered acceptable.

Results: The overall acceptable performance (100% PT score with the correct algorithm followed) was found to be 62% while 12% scored 80% and 11% scored between 20 to 60%. The rest 15 % were not considered as acceptable due to failure to adhere to the NHTA.Testing sites that participated in External Quality Assessment/Proficiency Testing(EQA/PT) schemes has shown better performance than those that did not participate in PT schemes with 70% and 56% performance, respectively (p=0.057). Though adherence to the NHTA, Training on HIV rapid testing, following SOP, were all positively associated with HIV rapid testing performances, all of them were not statistically significant.

Conclusion: Expansion of HIV diagnostic services at different points of care must ensure that appropriate quality assurance programs has been put in place. The lower level of PT performance together with problems observed during onsite evaluations calls for regular supervision, strong EQA programmes, adequate hands on training, and proper assignment of testing personnel.

Key words: *Acceptable Performance, dry tube sample, HIV testing sites, national HIV testing algorithm, proficiency testing*

1. Introduction

1.1. Background

The HIV/AIDS pandemic is still a global problem and ithas remained as a big challenge causing suffering especially in the SubSaharanAfrica.In 2018, globally, there were 37.9 million [32.7 million–44.0 million] people living with HIV out of whom around 26million found in Africa. From the total number of people living with HIV,36.2 million [31.3 million–42.0 million] were adults, and1.7 million [1.3 million–2.2 million] were children (<15 years). Of all people living with HIV,79% [67–92%] knew their HIV status and 23.3 million [20.5 million–24.3 million]were gettingantiretroviral therapy.[1].

HIV prevalence in Ethiopia is estimated to be 1.3% (95%CI 1.2%-1.5%) in the adult population. And it is also indicated that acces to HIV testing has been improved over the last decade[2].

HIV testing can be conducted for diagnosis, surveillance or blood screening and the selection of HIV testing approaches vary according to countries' policies. HIV counseling and testing is crucial for improving awareness and initiating treatment, care and other support services.People's knowledge of their HIV status through HIV testing servicesis also important for the success of prevention and control efforts on HIV. In SubSaharan Africa, it is estimated that less than 40% of persons know their HIV status[3,4].

Rapid diagnostic tests(RDTs) for HIV infection yield quicker test results.They also allow using capillary blood collected by a simple fingerstick procedure and therefore do not require venipuncture specimen collection. These features of RDTs has enabled health workers and other nonlaboratory staff perform HIV testing with high accuracy and reliabilityprovided that they get adequate training[5].

Expanding HIV testing services help in increasing case detection rate thereby providing information for programatic interventions. WHO recommends making HIV testing serviceavailable through a wide range of approaches, both in facilities and in the community.In addition to laboratory personnel, nurses,and Health Extension Workers (HEWs) in the community are key actors to HIV tesing service decentralisation[6].A recent analysis of national policies for HIV testing across 50 countries showed that 42% allowed lay providers to perform testing using RDTs (64% in African countries) and even more allowed lay providers to perform pre- and post-test counseling (56% overall and 80% in Africa) [7]

In rolling out of HIV RDTs in the community, quality assurance programmes to ensure the quality of community HIV rapid testing should also be implemented. Acheiving this task may be more challenging in community settings and the sensitivity and specificity of HIV point-of care testing may be affected by training and competency and the testing environments[6].

Quality Assurance is an integral part of sero diagnosis 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, the availability of trained and qualified personnel who perform rapid HIV testing being one of the most important factors for ensuring accurate and reliable results[7].To obtain reliable results and ensure the success of screening programmes that involve many healthcare workers (HCWs), it is not sufficient to use high quality reagents and simplemethods. It is also necessary to implement an external quality assurance system (EQA) to ensure proper performance,from sample collection to reporting of results, in each of the centres where the tests are used and in all the steps involved in the process[8].

External Quality Assessment Scheme (EQAS) refers to a system of objectively checking the performance of testing laboratories or centers at intervals by an external agency with the objective of attaining improved serviceto ensure trueness of results.The most common method of external quality assessment of a testing center's performance is proficiency testing in which a reference laboratory sends to testing sites a proficiency panel of specimens that are to be analyzed, and to be identified as HIV positive or HIV-negative[9].

Competency assessment, i.e. how well testing personnel are doing their job should be evaluated in order to reduce errors to a minimum. An evaluation of performance competencies of testing centers can be conducted through Proficiency Testing (PT). The PT for HIV rapid test provides a chance for participating health facilities to assess their gaps and learn to improve their performances there by produce accurate and reliable results[10].

The other method to conduct External Quality Assessment for HIV testing is On-Site Evaluation. This is a site visit which provides an opportunity to observe tester's performance under actual conditions including assessment of supplies, applications of basic procedures and safety conditions.

Serum and plasma specimens requiring stringent conditions for storage and transportation have been in use for PT programs and quality control (QC) purposes. This is expensive and difficult to implement in resource-limited settings. To overcome the cold chain transportation and PT panel delivery problems, a dried tube specimen (DTS) based PT are developed. These specimens are stable at room temperature for at least 1 month and can be rehydrated at the testing site for PT purposes. DTS are advantageous in that they are safer and less bio-hazardous than liquid specimens. In addition, the specimens are stable at temperatures expected in many countries, especially during storage and transport, and hence there is no need for maintaining an expensive cold chain. Once received at the testing facility, the specimens can be stored at room temperature for a few days without negatively affecting the integrity of the specimens [11].

The HIV testing must be done using accepted RDTs and following national HIV testing algorithm.To improve the quality of service delivery, all HIV RDTs used should have a sensitivity of at least 99% and a specificity of at least 98%, resulting in an overall positive predictive value of 99%. Selection of the rapid HIV tests and test algorithms to be used in testing and counselling services is a responsibility of national governments and is predominantly performed by health ministries and national AIDS control programmes [12].

WHO's standardized testing strategies are simple and low-cost, and they maximize the accuracy of HIV diagnosis. These testing strategies differ for populations with high (prevalence equal to or above 5% in the population to be tested) or low (prevalence below 5% in the population to be tested) HIV prevalence. In both instances, however, a series of three different assays – rapid diagnostic tests (RDTs), enzyme immunoassays (EIAs) or other supplemental assays – may be needed to establish an HIV diagnosis. An HIV testing algorithm describes the specific brands of assays used in a given HIV testing strategy[10].Accordingly, Ethiopia as a low HIV prevalence country has adopted the current National HIV Testing Algorithm (Figure1).

The use of rapid HIV antibody tests has been endorsed by the World Health Organization and adopted into national guidelines in many countries in sub-Saharan Africa. The expansion of point of care HIV testing undoubtedly requires attention and support to testing sites [13].The World Health Organization (WHO) requires the fulfillment ofquality-ASSURED**(A**ffordable, **S**ensitive, **S**pecific, **U**ser-friendly, **R**apid and robust, **E**quipment-free,**D**eliverable) criteria as a standard for point-of-care (POC) tests especially for healthcare facilities in resource-limited settings [14,15].

In view of expanding and improving the quality and safety of HIV rapid testing services, PEPFAR, in 2013 had introduced an HIV rapid testing quality improvement initiative (RTQII) in sevencountries, including Ethiopia. Consequently, 188 testing centers in 45 hospitals were selected to be enrolled in this initiative in Ethiopia in 2015 and 2016. The HIV RTQII in Ethiopia had a goal of expanding quality service on HIV rapid testing. Subsequently, facilities that were selected based on work load received limited support from PEPFAR through its partners where standard registration books for data recording was provided and training on HIV rapid testing was given for testing personnel[16].

The RTQIIis comprised of five key action areas [16]:

1. Policy Engagement: Creating enabling environment for policy development.
2. Human Resource Development: through training and certification of testers.
3. Proficiency Testing Programs: Monitor the quality of testing
4. Standardized Logbook: Monitor levels of uptake and coverage by entry in standardized patient registers.
5. Post Market Surveillance and Lot Verification: monitoring the quality of test kits and new kit lots procured and used within a given market, program, or country.

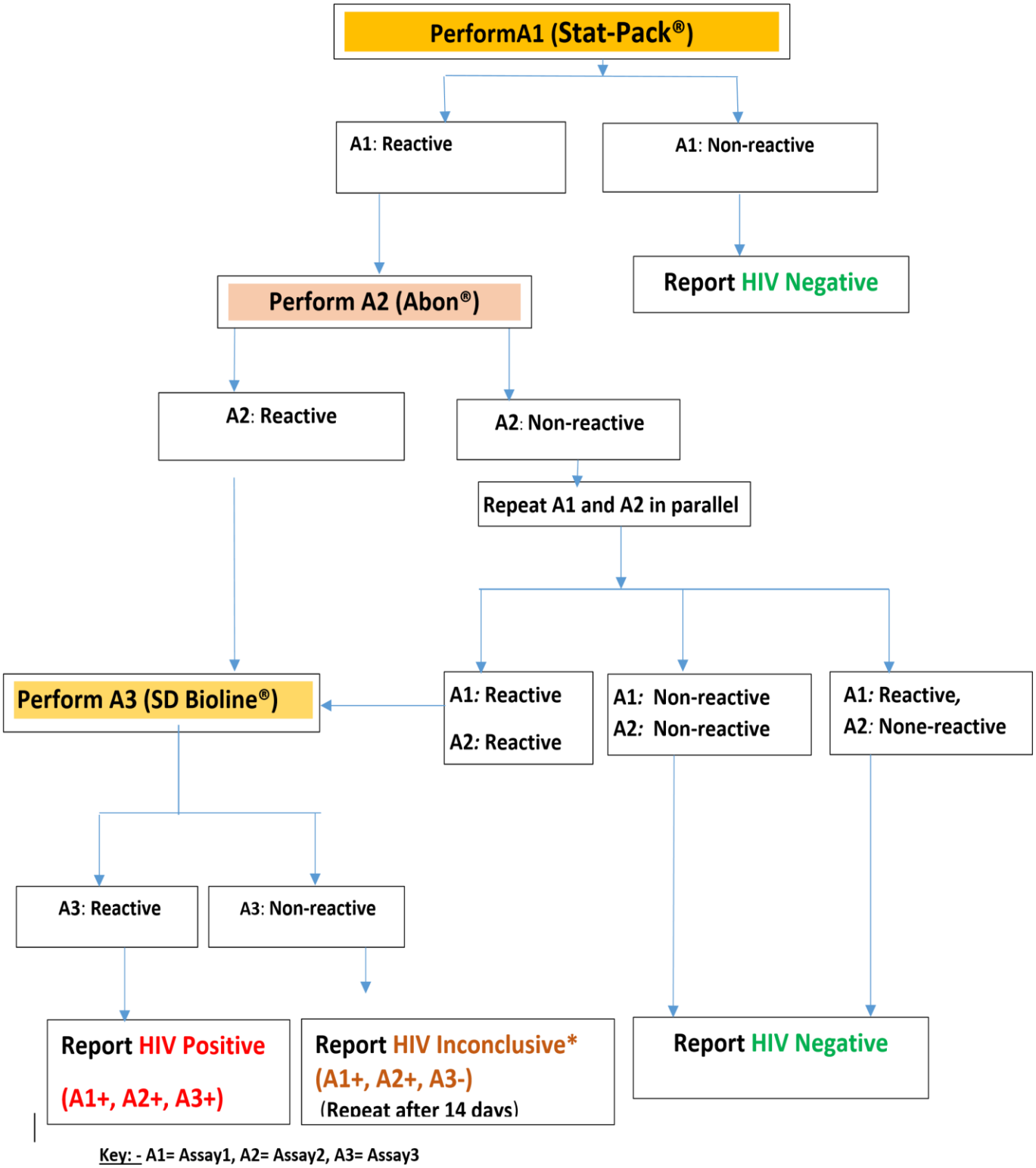

Figure 1; National HIV Testing Algorithm, Ethiopia, 2018[17]

1.2 Statement of the Problem

HIV testing is a crucial activity having significant impact on the prevention, treatment and care of citizens. And it is true that this service has been expanded to manysites in order to offer many people the chance to be tested. However, the effects of this expansion on quality of testing and accuracy of test results should be given due emphasis.

Poor quality HIV testing can arise from a number of factorsincluding: poor product performance, improper storage of test kits and supplies, clerical or transcription errors, user errors in performing the test and/or interpreting the test result. Lack of training, improper use of the testing strategy and/or algorithm, lack of supportive supervision and training, lack of standard operating procedures (SOPs) and poor documentation and record-keeping practices areamong the factors affecting quality service [4].

Recent reports suggest that misdiagnosis of HIV status is occurring in resource-limited settings. Recent policy analysis also suggests that only 20% of national HIV testing strategies align with WHO recommendations[2].An audit in three countries conducted by Médecins Sans Frontières found significant rates of false-positive diagnoses reported to individuals (2.6%–4.8%). Retesting all individuals diagnosed HIV-positive found that 10.3% had been misdiagnosed HIV-positive in the Democratic Republic of Congo and 7.1% in Ethiopia. In Malawi, during a three-month period in 2014, 7% of people previously diagnosed HIV-positive who were retested did not have a concordant HIV positive status and may have been misdiagnosed [3,7].

In Ethiopia, with regard to the performance of HIV Rapid testing, a cross sectional study representing testing sites' in most regions of the country, was not available.Thus, implementing quality assurance in HIV rapid testing service lacked information for realizing effective intiatives.

1.3. Significance of the Study

Quality management plan together with regular monitoring of HIV rapid testing service forms the basis for providing quality test results. In relation to the provision of quality HIV rapid testing services, it is appropriate to answer the question: "To what extent sites performing HIV rapid testing produce accurate results and how correctly they conduct the testing using the current algorithm?"

This cross sectional study was done to assess the performance of HIV testing sites and it was designed to be representativeparticipating sites in most regions of the country. Therefore, this study was initiated to assess HIV rapid testing performances and facility conditions of selected HIV rapid testing sites in Ethiopia.

The findings of this study will be very helpful to understand the level of performance of HIV rapid testing in the country. This study will also help identify the gaps and challenges in relation to HIV rapid testing services thereby creating a chance for effective intervention. Such assessment will be useful to give information to stakeholders as where to act in order to improve HIV diagnostic services in the country. Thus, the outcome of this study is believed to help make decisions to monitor, evaluate, and adjust the modalities of HIV diagnostic service in the country. Clients will also get appropriate care and management that originate from reliable test results. Finally, this study may serve as a spring board for further research work on the area.

2. Literature Review

2.1 Review on HIV strategies and Quality Assurance programs

The Federal Democratic Republic of Ethiopia revealed, in the Country's Health Sector Transformation Plan II, 2015-2020, that the country is commited to reduce new adult HIV infections by 50 percent by 2020 and to ending AIDS as a public health threat by 2030. In this regard a reduction of HIV incidence rate from 0.03 percent to 0.01 percent has been taken as one of the major indicators [18].

The 90–90–90 targets called for 90% of all people with HIV to be diagnosed, 90% of people with HIV diagnosed to receive ART and 90% of those on ART to have a suppressed viral load by 2020. In relation to this, of all people living with HIV, 79% [67-92%] knew their status, 62% [47-74%] wereaccessing treatment and 53% [43-63%] were virally suppressed in 2018.A complementing ambitious 95-95-95 plan targeting (95% of people living withHIV diagnosed, of whom 95% are on treatment, ofwhom 95% are virally suppressed), have recently been set for2030 [19,20]. However in order to acheive these targets, correct HIV diagnosis is important and all programmes providing HIV testing service must strive for accurate HIV diagnoses.

An HIV test result can have life-altering and lifelong consequences. Therefore, correct test results are crucial. Misdiagnosis of HIV status is too common and it was indicated that it rose as high as 10% in a study that retested people initially diagnosed HIV-positive [21].

External quality assessment (EQA) established by CDC as a cost-free voluntary program was implemented to monitor the performance of laboratories conducting HIV Early Infant Diagnosis (EID) from dried blood spots (DBS)in low to middle income countries since 2006.Ten blind DBS proficiency test (PT) specimens and 100 known HIV-positive and negative DBS specimens (to be used as internal controls) were shipped tri-annually to participating laboratories. It was indicated that mean PT test scores improved over time as demonstrated by the upward trend from mid-2006 to the end of 2012 (P0.001) and the increase in the percentage of laboratories coring 100%(P=0.003).The mean test scores rose over the past 10 testing cycles, ranging between 98.2% and 99.7%. Analysis of these test results suggested a positive impact of proficiency testing on the testing performance of the participating laboratories [22].

A proficiency testing program in China that had two components, an international and national, were started in 2006 and 2011 respectively. A total of 104 specimens were distributed over 13 international PT rounds, and 24 specimens were distributed over 3 domestic PT rounds. For the domestic PT program, there were 31 and 32 participating laboratories in 2011 and 2012, respectively. From among the returned results, 95.7% achieved a perfect score over 3 domestic PT rounds. Three (9.7%) laboratories in the initial round and one (3.1%) in the third round failed to attain a pass rating. Three of these four laboratories with failed rating were new PT participants. The 95% limits of agreement include 92.3% (96/104) and 91.7% (22/24) of all difference data from international and domestic PT, respectively [23].

HIV PT programme conducted from 2006 to 2017 in Haiti showed an average>90% HIV RDTs score across laboratories. However, one question capturing the challenges and limitations of the PT programme was how much of the quality of routine HIV RDT in the field is captured by the laboratories' PT scores. This is a concern since HIV RDTs are performed not only by laboratory technicians but also by nurses and aid-nurses that are usually not trained or have received limited on-the-job training [6].

In a study conducted in Kenya, 51 participants were selected from 7 sites to participate in proficiency testing. The sites comprised both private sector and institutions that do not participate in the National HIV referral Lab-PT scheme. Participants were provided with panels containing six samples to analyze using the current National HIV Testing Algorithm (NHTA). Results showed that 89% of the panels were correctly identified by the participants as positive or negative. Among the 11.0% errors, 74.2% were committed in one or more test result obtained while 12.9% committed in failure to follow NHTA.Root cause analysis revealed that the error committed by participants were as a result of failure to observe the kit manufactures' instructions and NHTA guidelines[24].

In Zambia, a proficiency testing study in HIV rapid testing was conducted through PT panels in two rounds, PT1and PT2. The majority of testers were lay counselors and nurses, together accounting for 77.9%and 72.3%of the testers in PT1and PT2 respectively. Nearly two-thirds (62.3%) of the participants inPT2 reported having received the standard HIV rapid testing training, while others (mostly lay counselors and nurses) had received other trainings such as PMTCT training (12.8%) and psychosocial counseling training (19.8%).A small proportion (5.0%) had no formal HIV testing

training at all, but reported having learnt to perform the HIV test from a supervisor or colleagues. The average overall accuracy level was 93.1% in PT1 and96.9% in PT2. Among sites that participated in both PT cycles, a significant upward difference was observed from PT1 to PT2 with overall accuracy levels of 91.4%and 96.7 %respectively. Comparing the two exercises, an improvement in accuracy level was seen among lay counselors (from 89.9% to 96.5%), and nurses (from 93.5% to 96.1%) while performance remained stable among laboratory personnel (98.7% vs. 98.7%)[25].

Another study was conducted in 2016, in relation to assessment of compliance with World Health Organization (WHO) rapid HIV testing quality elements in 485 rapid HIV testing points among204 health facilities located in 22 PEPFAR scale-up saturation districts across 14 regions of Tanzania. The RTQII audit process scores sites based on seven standards for rapid testing namely, personnel training and certification, physical facility, safety, pre-testing phase, testing phase, post testing phase, and external quality assurance (EQA) through proficiency testing (PT). Across the seven quality standards, none of the facilities performed at an expected level, although sites generally scored acceptable levels for physical facility and pre-testing. The performance gaps were much wider for the following standards: personnel training and certification (81%), EQA (70%), testing phase (61%) and safety (50%) [26].

A cross sectional study was conducted to assess quality assurance program of HIV testing in Addis Ababa Hospitals and Clinics, Ethiopia from May to October 2012. It was indicated that out of 20 assessed hospitals and clinics cases, 3(14%) laboratory personnel's who conduct HIV testing were found to have no training in HIV testing. Some laboratories 2 (10%) do not follow HIV testing algorithm. In this study it was indicated that there was lack of qualified human resources, failure to follow HIV testing algorithm and also poor participation in External Quality Assessment program. [27].

A cross-sectional study from Tigray, northern Ethiopia, which was conducted from April to June 2016 involved 145 testing points in sixty hospitals and health centers.From the participants,118 (81.4%) testing points scored 100%, 6 (4.2%) testing points scored 33%, 19 (13%) testing points scored 67% and 2 (1.4%) testing points scored 83%.The average performance of testing points varied from 89.6% to 99.1(Laboratory 99.1%, ANC 90.4%, TB 91.4% and VCT 89.6%).It was also noted that , among the participated testing points 41% had no designated area, 40% had no clean

water for hand washing and 51% had no national testing algorithm.This study demonstrated low (56%) coverage of HIV training among testers and itwas also indicated that 12% of the participants did not follow national test algorithm[28].

2.2 Dried Tube Samples(DTS)as a new approach for HIV PT

A novel and easy to use approach to conduct proficiency testing for HIV testing based on dried tube specimen (DTS) was developed that can help monitor the quality of HIV antibody testing in resource limited settings. DTS were prepared by drying 20 ul of specimen overnight at room temperature. The addition of a green dye (0.1%) made the DTS pellets visible without affecting the test results. Before testing, the DTS were rehydrated with 200ul of PBS-Tween buffer. A panel of 303 DTS samples (135 HIV positive and 168 HIV negative) was evaluated with two rapid tests. Sensitivity and specificity with the *Determine HIV-1/2* test were 99.3% and 99.4%, respectively, and with *OraQuick* were 98.5% and 100% respectively. Stability studies showed that HIV-specific antibodies in the DTS specimens were stable at 4^0c and 25^0c for 4 weeks, with only marginal decline at 37^0c and 45°Cover 4 weeks.The DTS-based PT program was piloted successfully in 24 testing sites in Kenya.And results demonstrated that the DTS is a simple to use, practical method to prepare and distribute PT panels to monitor HIV testing practices in resource limited settings [29].

2.3 Conceptual Framework

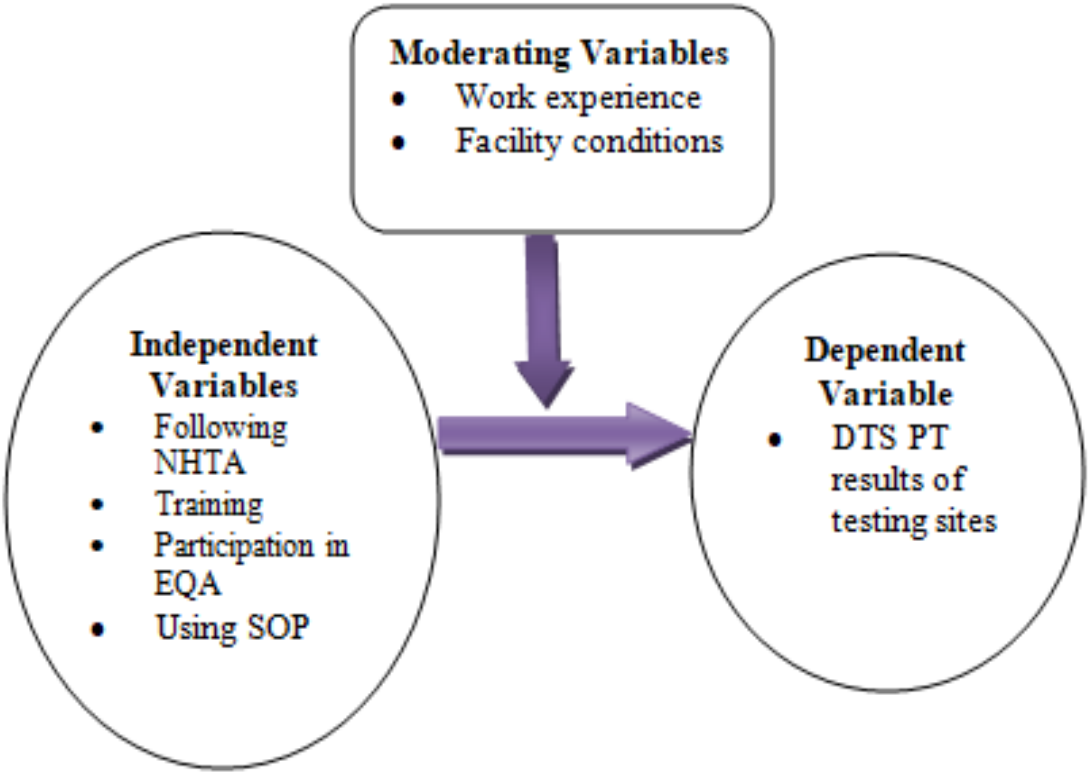

Figure 2; Conceptual Framework

3. Objectives of the Study

3.1 General Objective

To assess the performance and associated factors of HIV Rapid testing sites in RTQII enrolled health facilities through proficiency testing and onsite evaluation.

3.2 Specific Objectives

- To assess the competence of HIV rapid testing sites at RTQII enrolled health facilities using the current algorithm.
- To identify factors that affect HIV rapid testing quality at RTQII enrolled HIV testing sites and to identify areas for improvement.

4. Materials and Methods

4.1 Study Area

Ethiopia is a large country with a total surface area of almost 1.1 million square kilometers. Its topographic features range from 110 metres below sea level in the Afar Depression to the highest.peak, the Ras Dashen, at 4,550 meters above sea level. The ecological zones of Ethiopia are broadly divided into five zones based on elevation, each with its own rainfall pattern and agricultural production system : highland zones- locally identified as *Wurch, Dega* and *Wiena Dega*; and the lowlands zones known as *Kolla* and *Bereha*, i.e semi-arid and arid areas, respectively[30].

According to a 2017 estimate, Ethiopia has a population of approximately 105.35 million people, the second most populous country in Africa and the 13th most populous country in the world[31].

Ethiopia has nine regions and two city administrations. This study was conducted in five regions and the two City Administrations. It included a total of 159 HIV testing sites (HTS) in 41 Health facilities (HFs) as shown in the table below.

Table 1. Number of HTS and HF included in the study

No	Region	Number of Health Facilities	Number of HIV Testing Sites
1.	Addis Ababa	8	27
2.	Amhara	9	38
3.	Dire Dawa	2	9
4.	Harari	2	10
5.	Oromia	9	40
6.	SNNPR	5	15
7.	Tigray	6	20

4.2 Study Design and Period

Health facility based cross sectional study was conducted from August to December 2019 using non probability, purposive sampling method .

4.3 Population

4.3.1 Source Population

The source population is HIV testing sites (HTS) in all health facilities in five regions (Amhara, Harari, Oromia, SNNPR, and Tigray) and two City Administrations (Addis Ababa, and Dire Dawa).

4.3.2 Study Population

The study population for this research is HTS in all RTQII enrolled health facilities in five regions (Amhara,Harari, Tigray, Oromia, and SNNPR)and two City Administrations (Addis Ababa, and Dire Dawa).

4.4 Inclusion and Exclusion Criteria

HIV testing and ART sites that were enrolled in RTQII program were included in this study while HIV testing sites that were not enrolled in RTQII program were excluded.

4.5 Study Variables

4.5.1 Dependent Variable

HIV DTS PT performance of HIV rapid testing points in RTQII enrolled health facilities is the dependent variable.

4.5.2 Independent Variable

The independent variables included: use of HIV testing algorithm, training on HIV rapid testing, participation in EQA, and use of standard operating procedure (SOP). Other variables such as work experience, and facility conditions were considered as moderating variables.

4.6 Measurement and Data collection

4.6.1 Data collection procedure

Report forms containing results of DTS PT samples done by testing personnel together with completed checklists by each of the data collectors were submitted to the principal investigator at EPHI who entered the information into database for analysis. The preliminary data entry were reviewed, and checked through a data cleaning process using the Microsoft Excel program and Statistical package for social scientist (SPSS) version 23.

The sites were provided with five DTS PT samples (two HIV positives and three HIV negatives together with buffer solution, pipette, reporting forms, and printed instructions. DTS samples were transported to testing facilities in leak proof *nunc* tubes. DTS are stable for at least one month within a broad temperature range during storage and transport and hence do not require cold chain maintenance [32].

4.6.2 Sample Preparation and Verification

4.6.2.1 Sample preparation

The DTS PT panels were prepared and verified by the Principal Investigator and the EQA-PT provider team at the Ethiopian Public Health Institute (EPHI). A checklist, which was commented by professionals at EPHI and piloted in few selected testing sites in Addis Ababa, was also used for on-site assessment. Laboratory professionals who participated in the production of PT samples were all competent and had the experience of providing training in HIV rapid testing at the national level. Two positives and three negative DTS Panels for each testing site, constituting a total of 795 specimens with 318 positives and 477 negative were distributed.

Phosphate Buffered Saline (PBS) with PH 7.2 was prepared and distributed in to separate tubes and sent together with HIV DTS PT samples. Then plasma specimens were reconstituted by adding 7 drops of buffer and left overnight (12 hours) before testing (Annex V). A thorough mixing/tapping of the reconstituted samples was done before doing the test to maintain homogeneity of the sample.

Testing sites were expected to follow the National HIV Testing Algorithm (NHTA) while testing the five HIV DTS samples coded 1 up to 5. Supervising personnel had taken part in the reconstitution process to ensure that it is done properly and left overnight before testing. This activity was not considered as a variable in this study since the objective of the study was directed towards assessing the testing capabilities of HIV rapid testing sites.

4.6.2.2 Verification of HIV DTS PT samples

Taking all the necessary safety precautions, HIV DTS PT samples were prepared according to DTS PT sample preparation procedure (Annex IV).Characterized specimens with a combination of negative and positive reactivity for HIV were verified by the PI and EQA team at EPHI. After having prepared the required amount of plasma sample, a green food coloring dye was added to the plasma in a dilution of 1:1000,for example 1 µl of dye to 1 ml of specimen, and mixed by vortex to make a homogeneous colored mixture. Adding the green dye does not affect HIV test results but simply enables visualization of the small volume (only 20µl) of plasma at the bottom in each tube [29].

The 20µl of the colored plasma was transferred in to labeled small tubes and left open for overnight in a Bio Safety Cabinet to dry. The following day the tubes were capped after making sure that they are dried. On the following day, using simple random sampling method, a 5% of samples from each batch and each coded HIV DTS PT produced were selected for verification and reconstituted with buffer. On the following day, verification testing was done on selected samples and finally, status of each coded sample was documented and samples were made ready for dispatch to testing points.

4.7 Data Quality Assurance

4.7.1 Homogeneity

In order to ensure homogeneity and verification of HIV DTS PT , 5% of samples from each batch of HIV DTS PTduring production timewere tested and status of each coded sampledocumented before theDTS PT samples were dispached to testing sites.Result reporting form and standard checklist was used to collect feedback from testing sites.In order to maintain consistency and coherence, the principal investigatorprovided orientation to data collection team thatis composed of trained and skilled laboratory technologists.

4.7.2 Stability

Stability of the shipped PT materials was monitored by way of sending additional set of HIV DTS PT samples together with the DTS PT samples sent to testing sites. These additional samples were sent to go through all the conditions endured by the HIV DTS PT samples during transportation and were made to return back to EPHI where they were finally checked to give the expected outcome, i. e. their previous status. It was planned that if results differ from the expected outcome, feedback reports from sites that received the same batch of DTS PT samples would be excluded from the analysis. However, all returned samples proved to be stable as they yield the expected result after retesting up on return.

4.8 Data analysis and interpretation

HIV DTS Panel samples comprising five for each testing site with two positives and three negativeswere distributed. Accordingly, the overall evaluation panel constituted a total of 795 specimens with 318 positives and 477 negatives. Performance of correct result using the current algorithm was considered as acceptable performance. Each of the five panelswas given a 20 percent score totally making up 100 percent score. Any score less than 100 percent as well as failure to adhere to the NHTA was considered unacceptable performance.Data collected using checklist was also included to analyze factors contributing to acceptable performance by testing facilities.

All data were entered in to excel sheet and imported to SPSS version 23.0 for analysis, cleaned and coded. Thecontribution of different variables up on HIV rapid test performance was evaluated and calculated, differences and agreements between indicatorswere also assessed.Desriptive and summary statistics was done. Chi-square test of association was used to determine the association of each independent variable with the outcome variable.

4.9 Ethical considerations

The proposal of this thesis was reviewed and approved by Addis Ababa University, College of Health Sciences, Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences. This study was done to review HIV rapid testing performances of testing sites and was conducted as part of the National EQA program of the Ethiopian Public Health Institute (EPHI). HIV negative PT samples were obtained from the Ethiopian National Blood Bank (ENBB) based on agreement concluded between ENBB and EPHI whereas characterized HIV positive blood specimens were received from CDC-Ethiopia for the preparation of PT panels. In both cases samples were not traceable to their origin (donor).With regard to testing site or testing personnel, any information that has been associated to either of the two, has been delinked from the final data set.

4.10 Dissemination of Result

The first copy of the finding was submitted to Addis Ababa University, Department of MedicalLaboratory Sciences to be used as reference material for students and researchers. The other copies weregiven to the Ethiopian Public Health Institute and the Ministry of Health of Ethiopia so that theinformation can be used for program revision and intervention purposes.Finally the research outcome will be presented in different scientific seminars, conferences,workshops, andwill also be published onreliable scientific journals.

4.11. Operational Definitions

Dry Tube Samples:- Proficiency Testing panels for HIV rapid testing produced by transferring serum or plasma into tube that are stable at room temperature for at least one month, thus can be safely distributed to participants.

External Quality Assessment (EQA): A system for objectively checking the capabilities of testing sites by an external agency or facility. It allows participants to compare their performance against expected outcome.EQA includes on-site evaluation, panel testing, and blinded rechecking methods.

HIV Testing Sites (HTS): Point of care HIV testing centers at Health Facilities (Hospitals) that are selected as required by the conditions of health services at respective health institutions.

On Site Evaluation (OSE): Site visit to obtain a realistic assessment of the conditions and skills practiced at testing centers. It is ussually carried out by experienced personnel from a higher-level using a checklist. On-site evaluation is done as part of an ongoing EQA process.

Proficiency Testing: also referred to as Panel Testing, is the process of assessing the competency of testing sites by way of sending panels of well characterized specimens to testing sites for blind testing and evaluating their performances against the expected result.

Quality Assurance: is an ongoing set of activities that should be in place during the entire testing process to help ensure that the test results provided are as accurate and reliable as possible for all persons being tested.

Rapid Diagnostic Test (RDT): in vitro diagnostic of immune chromatographic or immune filtration format for the detection of HIV-1/2 antibodies.

National HIV Testing Algorithm (NHTA): the combination and sequence of specific assays within HIV testing strategies and used nationally to diagnose HIV infection, in this case: STAT PAK, ABBON, and SD- BIOLINE.

5. Results

5.1 Composition of Participants

A total of 159 HIV rapid testing sites in 41 Hospitals (HLs) participated in this study. HIV rapid testing sites in facilities enrolled in RTQII that qualified the inclusion criteria at the time of the study were taken as study sites. This included: Voluntary HIV Counseling and Testing (VCT), Prevention of Mother-to-Child Transmission (PMTCT), Provider- Initiated Counseling and Testing (PICT), Delivery ward, TB treatment centers, Emergency and Triage and others. With regard to the type and frequency of HIV rapid testing participant sites, it is illustrated below in Figure 3.

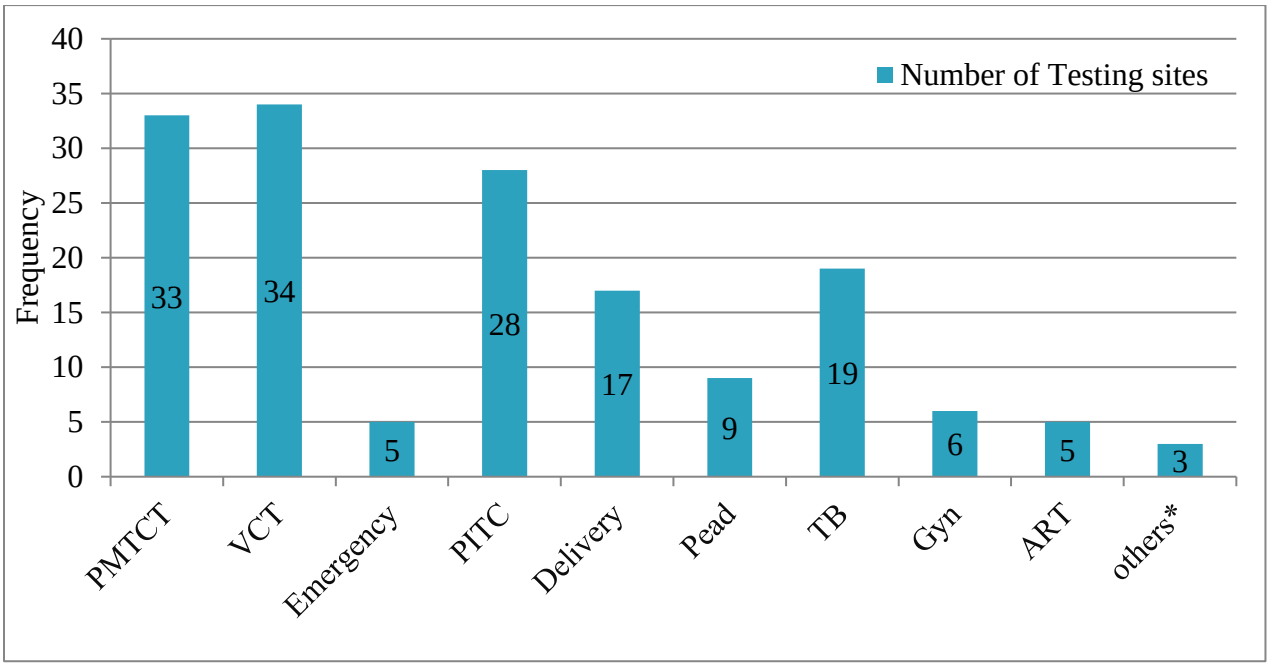

Figure 3.Composition of participant HIV Rapid testing sites in Ethiopia, August to December 2019 (n=159). Others*: OPD Triage, Medical Ward, Dermatology.

5.2 Performance of HIV testing sites

A 100% correct result for HIV DTS PT samples together with adherence to the NHTA was taken as acceptable performance. As shown in Figure 4, the overall acceptable performance by HTS was found to be 62% while 12% of HTS scored 80% and 11% of HTS scored between 20 to 60 percent. Though they made a correct final report of the HIV DTS PT samples, the rest 15 % were not considered as acceptable due to failure to adhere to the NHTA. Data collected during on-site visits using checklist was also used to study facility conditions at HTS. Hence it is addressed through descriptive presentations using tables and graphs.

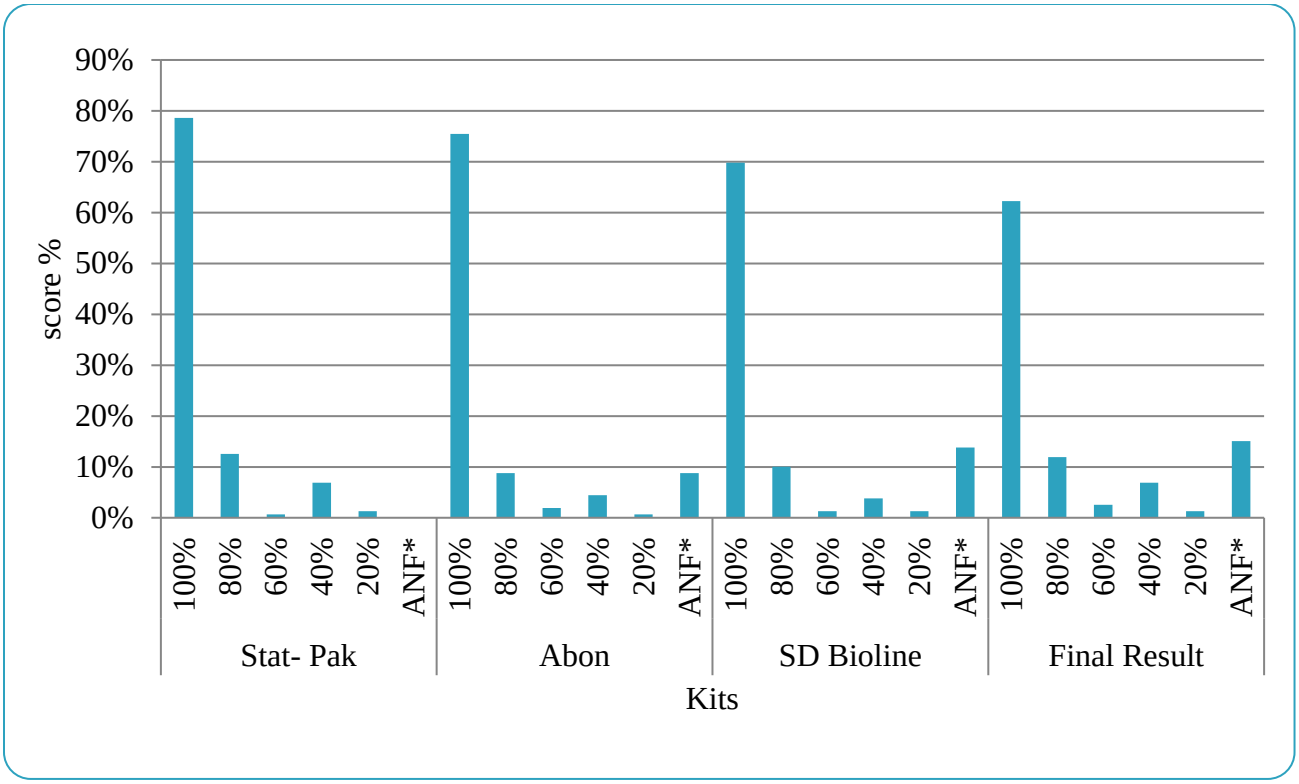

Figure 4. Testing sites' score (%) - each test kit with final status. ANF*: Algorithm Not Followed.

Assessment of the testing sites in relation to some parameters revealed that almost half (49.7%) of testers did not follow SOP, 60% did not have complete testing kits at work stations, 55.3% did not participate in PT in the last two years, 54.4% did not receive supervision visit from the facility's laboratory. The study also revealed that waste management and infection control practice was limited in some of the testing sites. With regard to training, 31% (50 out of 159) were not trained on the new algorithm as summarized in Table 1. Even among those who had taken the training, 77 % (84 out of 109) suggested that the training was not adequate to enable them acquire the necessary skills for efficient HIV rapid testing and emphasized the need for hands on training rather than a brief orientation or demonstration.

Table 2; Assessment of Different variables at RTQII enrolled HIV testing sites in Ethiopia. August to December 2019 (n=159).

Variable	Availability		Total
	No	Yes	
Test Algorithm posted	41 (25.8%)	118 (74.2%)	159
Tester followed SOP	79 (49.7%)	80 (50.3%)	159
Trained on the new algorithm	50 (31.4%)	109 (68.6%)	159
All test kits available at testing site*	96 (60.4%)	63 (39.6%)	159
Participation in EQA(PT) in the last two years	88 (55.3%)	71 (44.7%)	159
Receive supportive supervision from the facility's lab	86 (54.4%)	72 (45.6%)	158
Dedicated area(table or bench)for HIV rapid test	57 (35.8%)	102(64.2%)	159
Sink with water supply in testing area	56(35.2%)	103(64.8%)	159
Disinfectant for decontamination	49(30.8%)	110(69.2%)	159
Proper disposal of biological waste	38(23.9%)	121(76.1%)	159
Sharps container at testing site	22(13.8%)	137(86.2%)	159

*Most of the testing sites do not keep all three test kits at their workstations but resort to search for 2nd or/and 3rd kits from other centers in the facility at times of obtaining reactive test result on the 1st kit.

In this study the most important indicator of acceptable or accurate performance was participation in EQA(PT) with p=.057. Testing sites that participated in PT showed better performance than those testing sites which did not participate in PT with 70% and 56% performance, respectively. Though adherence to the NHTA, Training on HIV rapid testing, following SOP, were all positively associated with HIV Rapid testing performance with somehow increasing performance level, all of them were not statistically significant.

Testers were asked to respond about the difficulty or complexity of HIV rapid testing using the current algorithm by comparing it with the previous algorithm. Accordingly, 106out of 159 (66.7%) responded that the current algorithm was “more difficult” and of these, only 61% produced acceptable performance. Those who reported that the current algorithm was “comparable with the previous one” were 20(12.6%) and showed 70% acceptable performance while 14 (8.8%) responded that the current algorithm was “less difficult than the previous one” scored 79% acceptable performances proving a better score than the other two. The rest 19(11.9%) who gave a “don’t know” feedback had the least performance score of 53%. With regards to experience, testers having work experience more than 5 years produce better acceptable results (70% vs. 61%) than those with less than 2 years of work experience (Table 2).

As shown in Table 2, a remarkable proportion of those testing laboratories which did not practice internal quality control, produce unacceptable results than those who did (61.5% Vs 38.5%), though the difference did not reach statistically significant level.

Table 3; Result of Chi-square test of association between HIV testing performance and associated factors by testing sites in Ethiopia,August to December 2019 (n=159).

Indicators	Values	Performance Status				Total	Chi-square	P-value
		Acceptable		Not Acceptable				
IQC Conducted	No	52	38.50%	83	61.50%	135	0.233	0.629
	Yes	8	33.30%	16	66.70%	24		
Tester followed SOP	No	47	59.50%	32	40.50%	79	0.513	0.474
	Yes	52	65.00%	28	35.00%	80		
Trained on the new algorithm	No	29	58.00%	21	42.00%	50	0.564	0.452
	Yes	70	64.20%	39	35.80%	109		
Experience in years	< 2	25	61.00%	16	39.00%	41	1.451	0.484
	2-5	47	58.80%	33	41.30%	80		
	> 5	26	70.30%	11	29.70%	37		
All test kits available at testing site	No	59	61.50%	37	38.50%	96	0.067	0.796
	Yes	40	63.50%	23	36.50%	63		
Difficulty of the new algorithm compared with the old one	More difficult	65	61.30%	41	38.70%	106	3.928	0.269
	Comparable with previous one	14	70.00%	6	30.00%	20		
	Less difficult	11	78.60%	3	21.40%	14		
	Don't know	9	47.40%	10	52.60%	19		
Participation in EQA(PT) in the last two years	No	49	55.70%	39	44.30%	88	3.634	.057*
	Yes	50	70.40%	21	29.60%	71		
Receive supportive supervision from the facility's lab	No	52	60.50%	34	39.50%	86	0.195	0.659
	Yes	46	63.90%	26	36.10%	72		

Key: * marginally significant variable ($P \leq 0.1$)

Those who responded that the current algorithm was "more difficult" mostly complained about encountering a reactive test result with the first kit that usually proved to be non reactive using the second test kit. This, they said, has caused a simultaneous, repetition of the test using both 1st and 2ndtest kits as the NHTA dictates. This in turn creates not only wastage of test kits and delay in client's waiting time but also inconvenience and lack of confidence among both the client and the tester. However, feedback from data collecting supervisors witnessed technical problems by a significant number of testers. Among the most common problems mentioned were: failure to keep the appropriate reading time, adding wrong volume of sample and buffer. Also data collected through checklist showed that 50% of the testing sites did not follow SOP and only 46% of them received supportive supervision from the facility's laboratory.

6. Discussions

This study was conducted to assess the performance of HIV Rapid testing sites in RTQII enrolled health facilities through proficiency testing and onsite evaluation in the context of the current National HIV Testing Algorithm (NHTA). A total of 159 HIV rapid testing sites in 41 hospitals participated in this study. Out of 159 HIV rapid testing sites that participated in this study, the majority were VCT 34(21.4%) followed by PMTCT 33(20.7%), PITC 28(17.6%),TB 19(12%), and Delivery 17(11%).

In relation to assessment of proficiency testing results of participant HIV rapid testing sites (HTS), acceptable performance i.e. 100% PT score with the correct algorithm followed, was 62% while 12% of HTS scored 80% and 11% of HTS scored between 20 to 60 percent. Despite a correct final report of the HIV DTS PT samples, 15 % were not considered as acceptable due to failure to adhere to the NHTA. From chi-square test of association, the most important indicator that is associated with acceptable performance was participation in EQA (PT) with marginal significance of $p=.057(p\leq 0.1)$

In this study, non adherence to the NHTA refers to failure to perform a second test and a third test for a reactive result with the first kit, or reporting a result for the second kit or/and third kit while first kit test result of the PT sample proved to be non reactive. This is against the WHO current HIV testing strategy which is also adopted by Ethiopia as a low epidemic country (<5% prevalence) [10]. The decision to consider reports that did not adhere to the NHTA, regardless of the correctness of the final status, as unacceptable might have resulted in a relatively low proportion of acceptable

performance by testing sites. Thus, the overall performance when compared to results from similar studies seems to be lower. For example in a research done in Tigray, north Ethiopia, that in addition to point of care testing sites, they also included laboratory facilities as study participants. As a result 118 out of 145 (81.4%) participants scored 100% [28] where as in a study conducted at point of care sites in Addis Ababa, HIV rapid testing proficiency was reported to be 97.4% (at 95% C.I) [29]. The higher performance in the study from Addis Ababa [32] as compared to the current study could be, the later involved testing sites from all the participating regions which could have less access to supervision visits. Of note, testing sites which did not get supervision visits performed less in the current study signifying the importance of supervision visit.

Similarly the performance outcome of this study seem to be low when compared with a study conducted in Kenya where results of PTsamples from 51 sites showed 89.0%correct identification by participantswhile among the 11.0% errors, 74.2% were committed in one or more test result obtained [24].

Comparison of performance among testing sites indicated that VCT showed better performance, i.e.73.5% acceptable, seconded by PMTCT 66.7%, then PITC 60.7%, Delivery (52.9), and TB 52.6%, others having a lower score. With regard to failure to follow the NHTA by testing sites, particularly not being able to proceed the test using the second and the third kits when first kit yield reactive result, was mainly attributable to unavailability or shortage of second and/or third kits at testing site and facility level while the other reason was lack of awareness and attention. Failure to adhere to the NHTA by 15% of participants in this study seemed to be comparable with other similar studies. For instance, a study done in Tigray region showed that 12% of testing sites did not follow NHTA [28], and in Addis Ababa in two different studies, 10%[27] and 10.2% [32] of testing sites did not follow NHTA. Also in Kenya a similar study indicated a 12.9% failure to follow NHTA [24]. The reporting of no training by a third of the paricipants and absinceof supervision by more than haf of them in the current study might partly explain the failurity to adhere to the national testing algorithm.

In relation to facility conditions, 36% of testing sites did not have dedicated area (table/bench) for conducting HIV rapid test, 31% were without disinfectant for decontamination, 35% had no clean water supply for hand washing, while 14% did not have sharps container to dispose of sharps.Some of these findings seem to be comparable with findings from other studies.A study conducted on 145

testing sites in Tigray, indicated that 41% of testing sites had no designated area and 40% had no clean water for hand washing[28].In a similar study conducted in Addis Ababa,10.9% (29 out of 265)did not have separate area for HIV rapid testing, 22.3% had no disinfectant, and 35.5% had no clean water[32]. This finding underscores the need for close supervision of testing sites by the main laboratory of the health faclities as well as providing tranings on waste management and infection control.

As what is seen from onsite evaluations, it is worth to mention that some non standard practices were observed at testing sites. Sometimesduty assignment, i. e. who is supposed to perform HIV rapid testing at the time of visit, was not specified. Hence clinician that happen to be around and is willing to do the test may take the initiative and may perform the whole procedure or he/she maystarts the test and leave the site while other personnel continuestofinish it.Such practices may compromise the quality of the test result since there may be misunderstanding and communication gap between individuals involved in the testing process.In this regard VCT, ART,and TB sites had a good practice of having a responsible personnel assigned for HIV rapid testing service.

In relation to RTQII in Ethiopia, the program was short lived and seem to have fallen in short of providing continued support and proper evaluations of the rapid HIV testing service.

7. Strength and Limitation of the Study

7.1 Strength of the Study

The study provided comprehensive data by way of including large number of HTS in RTQII enrolled health facilities for the first time in the country. It also employed both PT and On-Site Evaluation at the same time as EQA method to assess rapid HIV testing performances of these sites.

7.2 Limitation of the Study

This study did not provide detailed information about the types and proportions of errors such as false positives and false negatives.

Some HIV rapid testing sites could not perform according to the national HIV testing algorithm(NHTA) due to lack of test kits at their facility.Thus they were discredited as producing unacceptable performance which likely contributed to lowered overall performance by participating sites.

8. Conclusion and Recommendation

8.1 Conclusion

The overall acceptable HIV rapid testing performance, i. e. 100% HIV DTS panel test score together with proper adherence toNHTA, by HTS was found to be 62% which is generally low. In relation to physical facility and organization,for example, having designated area for testing, availability of sink with water for hand washing, adequate supply of test kits and other necessary supplies, improvement is needed to ensure smooth HIV rapid testing service at the testing sites.

Participation in EQA/PT panels has shown a close association with acceptable performance with a marginal significance($p=0.057$). Those participated in PT schemes showed a better performance than those that did not participate with 70% and 56% acceptable performance respectively. Though adherence to the NHTA, Training on HIV rapid testing, following SOP, were all positively associated with HIV rapid testing performances, all of them were not statistically significant

8.2. Recommendations

The outcome of this study indicated that there is much to work on quality assurance activities including EQA/PT for HIV RDTs and such efforts should cover all testing sites especially point of care testing sites regardless of the setting.Therefore, based on the findings the following recommendations are forwarded:

- Expansion of HIV testing services at different points of care must ensure that appropriate quality assurance programs are in place. Accordingly, there is a need to work collaboratively to design efficient and sustainable strategies to continuously improve the quality of HIV rapid testing services across the country.
- Frequent and unusual changes in the National HIV rapid Testing Algorithm (NHTA) may create confusion and lack of understanding on the current working algorithm. Thus, decisions with regard to algorithm selection, adoption and change should follow a sound investigation and justification.
- The focus on expanding the number of testing sites should be balanced with the capacity to support and maintain quality in all of these sites. Otherwise, excess expansion of testing

sites by itself may lead to lowered follow up and poor supervision. Hence, it is much better to have limited and manageable number of testing sites within a facility.

- In general, implementation of regular supervision, strong quality assurance programs, assignment of competent personnel, adequate hands on training with proper orientation on NHTA, and good supply management should be major focus areas for improved HIV rapid testing services.

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Annexes

Annex-I: Information Sheet

Background Information: My name is Dereje Yenealem, I am currently doing a research to qualify for My Masters degree in*Diagnostic and Public Health Microbiology*at Addis Ababa University, College of Health Sciences,Department of Medical Laboratory Sciences.

Aim of the study: The aim of the study is to evaluate the performance of HIV Rapid testing using the current algorithm in RTQII enrolled testing centers in Ethiopia and produce information for potential areas of improvement.

Benefits of the study participant: The study can give opportunity for participants to know their strengths which they should keep up and identify gapsthat will be worked on for improvement. It will also helpto review and plan activities for quality assurance.

Risk and Complication of the participant: This study has no risk and complication in the participants.

Confidentiality: The information you provided will be kept confidential and will be used only for the study purposes.

Right to withdraw from the study: Participation in the study is voluntary and you have the right to participate or to withdraw any time and have the right to jump questions which is uncomfortable for you. However, your participation is important to full fill the study purpose.

I hope that you will be frank and honest in answering questions and testing the six HIV DTS PT samples. Do you agree to answer the questions on the checklist and test the samples to the best of your ability?

Yes ( ) No () If your answer is yes, please continue responding to the questions and testing the six HIV DTS PT samples.Thank you for participating with this important study.

Participant signature: _____ Date: _____

Annex II: Information Sheet (Amharic Version)

መግቢያ፡ እኔ ደረጀ የኔአለም እባላለሁ፤ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ በህክምና ላቦራቶሪ ዲፓርትመንትየሁለተኛዲግሪተማሪነኝ፡፡በአሁኑስዓትየመመረቂያማሟያፅሁፌንእየሰራሁእገኛለሁ፡፡

የጥናቱአላማ፡የጥናቱአላማከላቦራቶሪ ባለሙያ ውጪ በተለያዩ የጤና ተቋማት የሚሰጡየኤች አይ ቪምርመራ አገልግሎቶችንብቃታቸውንመፈተሽእናየአሰራርክፍተቶችን በመለየትአቅምንማሳደግያ መንገዶችን መጠቆምይሆናል፡፡

የጥናቱተሳታፊዎችጥቅም፡ከላቦራቶሪ ውጪ በተለያዩ የጤና ተቋማት የኤች አይ ቪምርመራ ላይ የሚሰሩ ባለሙያዎችብቃታቸውምንደረጃላይእንዳለለማወቅይረዳቸዋል፡፡ያጋጠሙክፍተቶችንበመጠቆም የአገልግሎት ጥራትከፍእንዲል ይረዳል፡፡የኤች አይ ቪ ምርመራና አገልግሎት ፕሮግራምላይለሚሰሩባለድርሻአካላትተጨባጭየሆነመረጃየሚሰጥይሆናል፡፡

ሊያጋጥምየሚችል ጉዳት፡በጥናቱላይየሚሳተፉባለሙያወችላይየሚደርስምንምአይነትጉዳትየለም፡፡

ሚስጥራዊነት፡እርስዎየሚሰጡንመረጃበሚስጥርየሚያዝእናለጥናቱአገልግሎትብቻየሚውልይሆናል፡፡

በጥናቱላይያለመሳተፍመብት፡በጥናቱላይለመሳተፍሙሉበሙሉበበጎፍቃደኝነትላይየተመሰረተነው፤

አለመሳተፍምሆነጥናቱንበፈለጉትስዓትማቋረጥይችላሉ፤

ነገርግንየእርስዎበጥናቱላይመሳተፍየተፈለገውንውጤትለማግኘትበጣምጠቃሚነው፡፡

በግልፅነትእናበታማኝነትጥያቄዎችንእንደሚመልሱልንእንዲሁምየምንሰጥዎትንየኤች አይ ቪ ናሙናዎች መርምረው ውጤቱን እንደሚሰጡንተስፋእናደርጋለን፡፡

ጥያቄዎችንለመመለስእናየምንሰጥዎትንየኤች አይ ቪ ናሙናዎች መርምረው ውጤቱን ለመስጠት ፍቃደኛ ነዎት? አዎ ( ) አይደለሁም ( )

መልስዎአዎከሆነ፤እባክዎትንእነዚህንጥቂትጥያቄዎችበመመለስእንዲሁምየሰጠንዎትንየኤች አይ ቪ ናሙናዎች ማከናወንይቀጥሉ፡፡

በዚህጠቃሚጥናትውስጥስለተሳተፉልንበጣምእናመሰግናለን፡፡

የጥናቱተሳታፊፈርማ፡---------- ቀን፡----------

Annex III: HIV Rapid Testing SiteAssessment Checklist

Facility Name _____ Testing Point (Name): _____
Assessment Date:_____
Assessment done by_____

Objective of this assessment is:

1- Identification of gapswith respect to performing HIV rapid test and share findings with those responsible for undertaking the necessary interventions.

		Y	N	NA	Comment
1.	Is there shortage of test kits for the last one year at testing site?				
2..	Is storage condition for test kits properly maintained according to manufacturers' recommendations?				
3.	Does the testing point only use nationally approved HIV kits for testing?				
4.	Is test Algorithm posted in a way that can easily be seen by testing personnel?				
5.	Are all test kits , listed in the algorithm,available at testing point?				
6.	Are buffer solutions available and appropriately used for specific test type?				
7.	Is the correct procedure posted in a way that it can easily be read by the tester?				
8.	Are IQC samples performed periodically? (See evidence)				
9.	Has testing site participated in EQA(PT) in the last two years? (see evidence of participation)				
10.	Are testers certified for HIV rapid testing ?(see evidence)				
11.	Does the tester check the expiry date of test kits before use?				
12.	Has the tester followed SOP during panel testing? (If not, describe the reason)				
13.	Has the tester labeled the test device before doing the test?				
14.	Has the tester added the correct volume of sample on to the device?				
15.	Is appropriate buffer and buffer volume used for the Specific test kit?				
16.	Has the tester made the reading at the appropriate time?				
17.	Does the tester keep records of tests done ?				
18.	How many years has the tester been performing HIV Rapid Testing?				
19.	Has the tester received training on HIV Rapid Testing on the previous algorithm?				
20.	Is tester trained on the new algorithm?(If No, escape Q.No 22-24)				
21.	Was the training on the new algorithm satisfactory and effective? (ask the tester's opinion) If No, Why?				

22.	How difficult is interpretation of result using the new algorithm compared to the previous one (old algorithm)					
23	Does the testing site receive supportive supervision from the facility's laboratory?					
24	a)Is the testing place a separate dedicated room, for HIV Rapid testing service only?					
	b)If the testing site shares the same room where other activities are performed, is there a dedicated table for doing HIV rapid test?					
25.	Is there a sink with water supply for hand washing at testing place?					
26.	Is there sharps container in use?					
27.	Is there adequate supply of PPE (gloves,Gown, & disinfectant)?					
28	Is there a disinfectant for decontaminating working area?					
29.	Is biohazard waste cleaned-up and disposed appropriately?					
30.	How difficult is the new algorithm to perform compared to the previous one (old algorithm)	More difficult	Comparable with the previous	Less difficult	Don't know	
31.	What is the professional background of the tester at the Testing Point?	Nurse	HEW	Community Councilor	Lab.	Other (specify)

Annex IV:Preparation of Dried Tube Specimens (DTS) for Proficiency Testing

SUPPLIES

- 2.0 ml conical bottom sarstedt tubes (catalog # 72-694-007;www.sarstedt.com)
- Green food coloring dye (Kroger brand)
- Pipettes those are capable of multi-dispensing (Rainin EDP3 pipette, catalog # E3-20).
  - ✓ Pipette tips
  - ✓ Disposable transfer pipettes (Samco Transfer pipet catalog number282)
- Freezer boxes
- Tube racks
- Cryo labels
- Storage bottles
- Zip Lock bags
- Labels

SPECIAL SAFETYPRECAUTIONS

- ✓ Wear protective clothing while handling DTS
- ✓ Handle DTS as if capable of transmitting an infectious agent.
- ✓ Do not interchange vial caps; this will lead to cross contamination of specimens.
- ✓ Leave the DTS in the Biosafety cabinet (BSC) for overnight drying of the specimen.

PROCEDURE

Obtain rejected plasma units from local blood bank of different HIV reactivity, including some HIV negatives. Initially acquire >10 units to build specimen inventory

Transfer plasma from the bag to a clean storage bottle. Store plasma at 4°C until further testing has been conducted.

Regardless of status given by the blood bank, the specimen reactivity should be verified by the laboratory that is responsible for providing the PT panel.

Plasma specimens should be characterized with respect to their HIVstatus by all HIV rapid tests, ELISA, and western blot (if available) based on country specific algorithm.

Rapid Test: Test plasma specimens with all commonly used HIV rapid tests in thecountry.

- ELISA: Test all samples on all ELISA procedure used in the country.
- Western Blot: If available western blot banding pattern should also be established for HIV positive specimens.

Preparation of PT buffer(PBS/Tween-20)

- Dissolve one foil pouch of phosphate buffered saline with tween 20, pH 7.4 in 1 L of de-ionized water. It will yield 0.01 M PBS; NaCl 0.138 M; KCl 0.0027 M; Tween 20 – 0.05%; pH7.4.
- Filter it through 0.2 µ filter flask.
- Prepare 1.8 ml aliquots in pre-labeled 2 ml screw capped tubes.
- The label on tube should include the following:

Identify the tube as "PT buffer"

Set an expiration date of 1 year after you prepare

Preparation of DTS

- Create a panel of 5 samples from the characterized specimens with a combination of negative and positive reactivity for HIV.
- Carefully blind the panel assigning a new ID to each of the 5 member panel. For example DTS-A1 to DTS-A5. Ensure there is a link of the original ID and new ID.
- Label each tube with appropriate new ID.
- Depending on the number of labs enrolled in the PT program, prepare 10 to 20 extra sets

- Prepare a 1:1000 dilution of green dye to specimen. For example add 1 µL of dye (food coloring) to 1 mL of specimen. Vortex the specimen to mix thedye.
- Prepare DTS by transferring 20 µL of colored serum/plasma specimen to sarstedttube.
- Ensure to aliquot each specimen in properly labeled tubes. Aliquot only one specimen at a time to avoid any possibility ofmixing.
- Leave the tubes uncapped in a BSC and let it dry overnight at room temperature. Make sure different specimens are kept in separate racks in aBSC.
- The following day recap eachtube making sure that the specimens have dried completely.
- A visible colored pellet is formed towards the bottom of thetube.
- Capped DTS are kept at 4°C until ready for shipment to the participatinglaboratories.

Packaging PTpanels

- Prepare PT panels for shipments to include thefollowing:

one member of eachpanel
one vial of PTbuffer
two plastic transfer pipettes (dropper)
one page of instruction sheet/hand out
one page of reporting forms each

- Put all the contents in labeled ziplockbags.
- The bagged PT panels can be stored at 4^0 C till shipment or delivery to testingsites

Result

- Collect report from all participatinglaboratories.
- Enter data in the excel spreadsheet
- Analyze the data using the PT spreadsheet
- Send the final report to all the participatinglaboratories.
- Follow up with a supervisor for those laboratories who donot receive a 100% passinggrade.

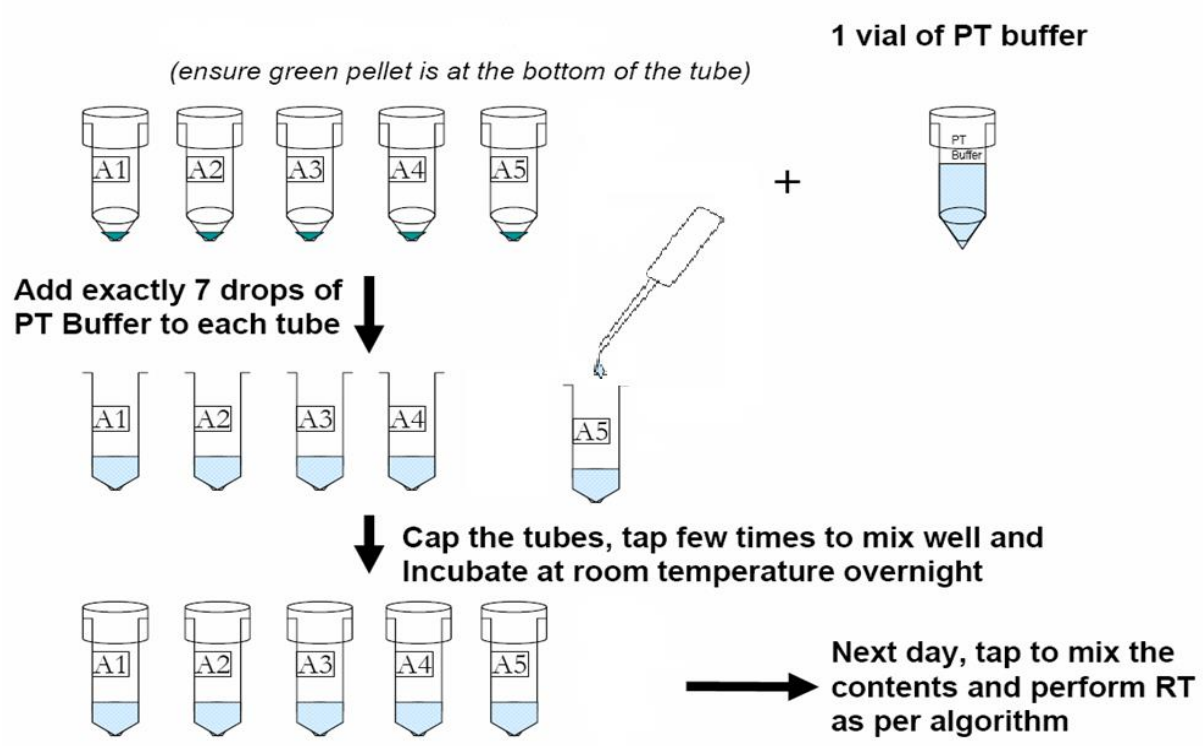
Annex V: Instruction for HIV DTS Panel test specimen re-constitution

- **Re-constitution of DTS**

a. Tap the tube gently before opening the cap to ensure that the colored pellet falls to the bottom of the tube.

b. Dried tube specimen is re-constituted one day prior to testing.

c. Using the dropper provided, add 7 drops of PT buffer (equal to 200 µl) to each DTS to be tested. Cover the tube, tap gently and incubate for minimum of 2 hours for same day testing, or overnight at room temperature for next day testing.



d. Before testing mix the specimen by gently tapping the tube.

e. Test the re-constituted DTS with HIV rapid test kit as that of you do for patient specimen testing.

f. Report the results using report form before the Deadline.

Annex 6: HIV Rapid Test DTS PT result reporting Form

Date: August 2019				
Facility Name		**Performed by**		**Signature**
Testing Point Name				
Date Samples Received		**Result & data collected by**		**Signature**
Date of reporting				
Test Kit Name				
Expiry Date				
Lot Number				

DTS PT Sample Code	**TEST 1 RESULT**	**TEST 2 RESULT**	**TEST 3 RESULT**	**FINAL RESULT (HIV STATUS)**

Remark:

_____
_____
_____
_____
_____.

Declaration

This is to certify that this study entitled "**Proficiency Testing and Onsite Assessment of HIV Rapid Testing sites at Health Facilities enrolled in HIV Rapid Test Quality Improvement Initiative (RTQII) in Ethiopia"** is an original work by the under signed Dereje Yenealem and has been submitted in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology). I have communicated to my advisors and all stakeholders involved in the study. I have provided progress report to my advisors and seek the necessary advice and approval.

Name of the student: **Dereje Yenealem (B.Sc)**

Signature: _____

Date: _____

Approval by Advisors

Name of advisor: **Aster Tsegaye (PhD, Associate Professor of Immunology)**

Signature:_____

Date:_____

Name of advisor: **Mr Kassu Desta (M.Sc, PhD Candidate, Associate Professor of Medical Microbiology)**

Signature:_____

Date:_____