

Pilot implementation of GeneXpert HIV-1 qualitative assay in selected health facilities: lessons learned from Ethiopia

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Abstract

Background: Access to Early Infant Diagnosis (EID) using the conventional DNA PCR method is challenging because it requires technical expertise and has long turn-around time for results. Innovative point-of-care HIV testing could bridge this gap. This study aimed to verify the performance of Point of Care (POC) GeneXpert HIV-1 Qualitative Assay POC for HIV-EID.

Methods: This study was conducted from May 2017 to April 2018. Initially, the performance of the GeneXpert HIV-1 Qualitative assay POC method was verified by comparing with the conventional (Roche CAP/CTM HIV-1 Qualitative PCR) method; this was followed by a baseline assessment of the pilot implementation of the assay, which had two categories. The first pilot was EID testing in eight hospitals in different regions of the country as a standalone testing for EID. The second part of the pilot was integrated to TB/EID testing in two hospitals and one health center, using the existing GeneXpert devices being used for MTB/Rif testing. Data was analyzed using SPSS version 26 (IBM Corp., Armonk, NY).

Results: Overall, 1,216 HIV-1 exposed infants were included in the study out of which the positivity rate was 48 (4%) using the GeneXpert HIV-1 Qualitative assay. The sensitivity and specificity of the GeneXpert HIV-1 Qualitative assay was 100% and accuracy was also 100% (95% CI, 97.3-100), with an overall error/invalid rate of 6.8% for Xpert HIV-1 Qualitative assay. Out of 1,216 samples, 993(62.6%) were tested with Xpert HIV-1 Qualitative assay, and the results were delivered within the same day. Turnaround time (TAT) was significantly lower in Xpert POC testing compared with CAP/CTM conventional testing methods. TAT predictor analysis showed that, almost 50% of the delay occurred from sample collection at the patient care setting until it reached the central testing laboratory.

Conclusion: This study showed excellent diagnostic performance of Xpert HIV-1 Qualitative assay compared with the PCR method. Additionally, the pilot assessment showed that Xpert HIV-1 Qualitative assay improved access to same day testing of HIV Exposed Infants (HEIs) and facilitated to put them on ART as soon as possible, if they tested positive. Therefore, since Xpert HIV-1 Qualitative assay can offer significantly improved care for HEI, strategic addition of GeneXpert POC testing to diagnostic algorithms to improve EID and treatment is recommended.

Key words: HIV-1, EID, GenXpert, Qualitative Assay, POC, PCR

Introduction

HIV continues to be a major global public health challenge. In 2020, there were 37.7 million people living with HIV (PLHIV) and, among these, 36.2 million and 1.5 million were adults and children under age 15, respectively (UNAIDS 2021). According to the 2016 Ethiopian Demographic Health Survey (EDHS), national overall HIV prevalence was around 1 % and there was variation in regional estimates. In Ethiopia, the prevalence of HIV is characterized by a low intensity mixed epidemic (generalized and concentrated) (EDHS 2016). Despite different interventions undertaken by the government, the mortality rate among HIV infected children remains higher than that, in the

general pediatric population. Previous studies conducted in Ethiopia reported that the mortality rate in HIV-positive children receiving ART ranged from 1.4 deaths per 100 child-years of observation (Gebremedhin et al. 2013), to four deaths per 100 child-years of observation (Koye et al. 2012). These studies also reported that many factors significantly increased the risk of mortality in this population. For example, baseline anemia (Hgb < 10 g/dL) (Gebremedhin et al. 2013; Koye et al. 2012), low CD4 count (Koye et al. 2012), advanced WHO clinical disease staging (III and IV) (Koye et al. 2012, Gebremedhin et al. 2013), poor ART adherence (Ebissa et al. 2015), and malnutrition (Mulugeta et al. 2022) were reported as factors

significantly increasing the risk of mortality in HIV-positive children receiving ART. HIV-positive and untreated children are at the highest risk of mortality within the first three months of life, and up to 50% die within two years (Violari et al. 2008). The World Health Organization (WHO) recommends early testing by six weeks of life and immediate ART for all infected infants younger than five years (WHO 2018).

The major limiting factor to successful early infant diagnosis (EID) is the long turnaround time (TAT). HEI testing algorithm in Ethiopia includes mother's acceptance of infant testing, specimen collection, transport, and laboratory testing, communication of results to healthcare providers and caregivers, and linkage to care and treatment, including opportunistic infection prophylaxis and ART for all HEI (Ciaranello et al. 2011). These steps are prone to delays and patient loss to follow-up (Ciaranello et al. 2011; Tiam et al. 2017; Sutcliffe et al. 2014). Traveling long distances for clinical care that requires logistics and resources are barriers to successful HIV treatment (Lankowski et al. 2014).

In 2016, the WHO prequalified, Xpert HIV-1 Qualitative assay on the GeneXpert instrument manufactured by Cepheid AB, as a near point-of-care (POC) diagnostic test for the detection of HIV RNA in whole blood samples. This POC technology is a cartridge-based, real-time reverse transcription polymerase chain reaction (PCR) qualitative assay performed on a benchtop instrument that is suitable for operation in the clinic setting and does not require extensive technical training (WHO 2016). POC technology allows for testing closer to the patient, particularly in resource-limited settings (RLS) (Drain et al. 2014). To achieve the 90-90-90 targets, in addition to strengthening the conventional laboratory testing, pilot and scale-up of POC GeneXpert HIV-1 Qualitative assay for EID was found to be critical by strategic placement of these tests within the existing laboratory system to provide the greatest access to infants and the most impact on the Prevention of Mother to Child Transmission (PMTCT) program (Ndoye et al. 2022).

The utilization rate of the existing conventional EID testing capacity in Ethiopia is low, partly due to logistic challenges for the transportation of specimens to centralized laboratories. Moreover, prolonged TAT observed in delivery of the results to the sites hinders early initiation of treatment (Kebede et al. 2016). Performance evaluation and then piloting of POC GeneXpert HIV-1 Qualitative test was believed to help guide widespread implementation of this assay. The aim of this pilot implementation was, therefore, to assess the feasibility of HIV-EID POC testing using GeneXpert and to examine all aspects of program management in rolling out POC EID testing.

Methods

Study setting and study area: In this study, the GeneXpert near-POC platform using the Xpert HIV-1 Qualitative assay for early infant diagnosis was implemented on a pilot scale in Ethiopia. Eight standalone GeneXpert platforms were introduced at eight government health facilities for standalone EID testing at six weeks, and in three facilities, integration of EID testing on existing TB devices was piloted. This one-year study which was conducted from May 2017 to April 2018, was initiated by a baseline assessment of the performance of the GeneXpert HIV-1 Qualitative test using conventional Roche CAP/CTM HIV-1 qualitative PCR as a reference test. This was followed by a pilot implementation which had two categories. The first was, EID testing through the purchase and distribution of eight GeneXpert devices and heat block in eight hospitals in Addis Ababa City Administration, Oromia, Tigray, Amhara, Southern Nations, Nationalities and Peoples Region, Dire Dawa, and Gambella regions. The facilities were Zewditu, Weliso, Maychew, Woldia, MizanAman, Dilchora, Shashemene, and Gambella hospitals as standalone testing for EID. The second was for the evaluation of integrated TB/EID testing in two hospitals (Bishoftu and Ras Desta hospitals) and one health center (Addis Ketema HC), using the existing GeneXpert devices being used for MTB Rif testing. The integration considered the case loads of TB or HIV exposed infants in the institution and based on the pre-assessment; these selected facilities did not have more than one exposed infant per day. Therefore, the integration did not affect the existing TB testing or the new EID testing performance in the facilities.

Each of the eight testing sites and the integration sites (Bishoftu, Ras Desta hospitals and Addis Ketema HC) were networked with an average of 2-11 facilities (facilities with no GeneXpert system and send DBS to the integration sites for testing) based on their GeneXpert MTB/Rif testing network. Networking was not the objective of the pilot, but these facilities took the initiative of informing their catchment health facilities to send DBS samples for EID testing to them.

Pilot site selection: Pilot facilities were collaboratively selected by the Maternal and Child Health (MCH) Directorate of the Federal Ministry of Health (MOH Ethiopia 2018), Ethiopian Public Health Institute (EPHI) and Regions to establish POC testing systems at these sites. The pilot sites were selected based on their testing volume, their distance from conventional DNA PCR testing laboratories, their ability to conduct all aspects of Xpert HIV-1 Qualitative testing and their capacity for managing the pilot.

Baseline assessment: A one-year baseline data on the conventional DNA PCR testing were collected from these sites using a standardized checklist and the data includes; date of DBS sample collected, date DBS sent to regional laboratories for PCR testing, date PCR test done in the regional laboratory, date DBS result received by the referring health facility, date result received by the infant families, DBS result of the infant negative/positive, date ART initiated, if positive and status of the infant alive/dead and finally TAT calculated. The sources of data were registers from PMTCT, ART clinic and laboratory. Patient charts were also used to triangulate and validate data to calculate TAT of the time between DBS collection from PMTCT clinics, the time sent to the laboratory and the time result was received by the PMTCT clinic and the mother/caregiver.

Verification: After providing appropriate training for laboratory professionals on Xpert HIV-1 Qualitative assay, an initial verification procedure was carried out to validate the performance of each

pilot site and each test operator. To carry out this procedure twenty HIV positive and negative DBS samples, prepared and tested using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Qualitative Test, v2.0 assay at the EPHI HIV Molecular Laboratory, were distributed to all pilot sites to be tested by trained laboratory professionals using Xpert HIV-1 Qualitative assay.

Pilot implementation of Xpert HIV-1 Qualitative assay: After the initial verification step, pilot implementation was started in all 11 selected facilities. GenXpert HIV-1 Qualitative assay testing cartridges, logbooks, flowcharts, SOPs, job aids and other materials, specifically designed for the implementation pilot, were provided to the pilot sites before actual testing was initiated (Figure 1). In the implementation phase actions were taken immediately based on the national antiretroviral therapy (ART) guideline, i.e. after result received by PMTCT/ART/inpatient/nutrition departments, anti-retroviral treatment was initiated immediately the same day (MoH 2018).

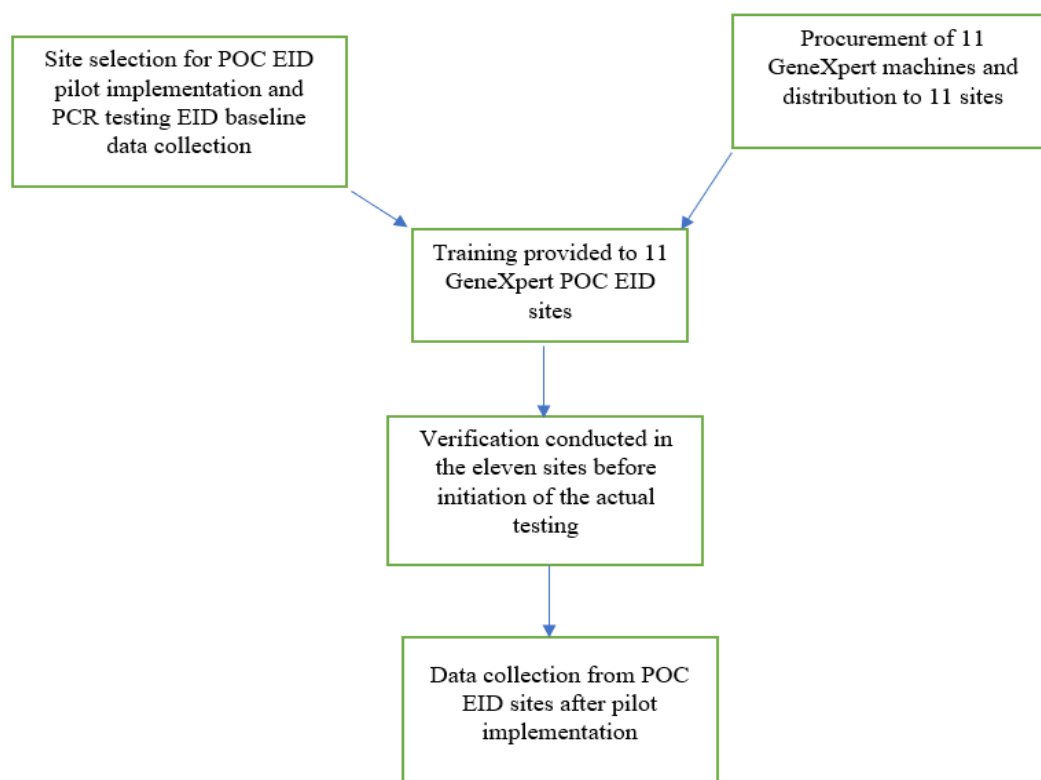


Figure 1: Flowchart of data collection process

Data collection tools and procedure: The same data collection procedure and similar periods as for the baseline assessment was followed during the implementation of Xpert HIV-1 Qualitative assay testing in the pilot facilities. Data were collected from PMTCT and ART clinics, and Laboratory registers specifically designed for the implementation pilot. The collected data were

entered into Microsoft Excel. To assure data quality, data triangulation was applied using available data sources. Since it was a pilot implementation, there was no sample size calculation.

Quality assurance: Two test operators from each site selected for the pilot were trained both on theoretical and practical aspects of Xpert HIV-1 Qualitative testing and were required to pass an

individual certification before administering routine testing in the implementation pilot. In addition to the laboratory professionals, one staff from each of the PMTCT, paediatric inpatient and nutrition units were also given training on sample collection and transportation in addition to the systems training. Weekly follow up through telephone and monthly site level supportive supervision was conducted. Based on the supervision findings, feedback was provided, and the implementation of action items was followed.

Data analysis: All POC results were entered into a Microsoft excel database (Microsoft Corporation, Redmond, WA, USA). Data was analysed using SPSS version 26 (IBM Corp., Armonk, NY). Participant characteristics were presented by use of frequencies and percentages for categorical data. Sensitivity and Specificity (with 95% Confidence intervals) of GeneXpert HIV-1 Qualitative EID POC were compared with Roche CAP/CTM HIV-1 qualitative PCR, which was taken as the reference testing method.

Ethical clearance: The study protocols and data collection tool were reviewed and approved by the Ethiopian Public Health Institute Institutional Review Board (Ref. Number SERO-071-12-2016). In the pilot implementation phase, written informed consent was obtained from every participant. In the

case of minors (HIV exposed infants), written informed consent was obtained from parents or guardians. The baseline data were collected retrospectively from medical records, personal identifiable information such as name, medical record number (MRN) and unique ART numbers not included in the data analysis/report forms, i.e. these information accessed only by healthcare workers and key study staff.

Results

Diagnostic performance of GenXpert HIV-1

Qualitative assay: The verification result showed that, 65 (44.2%) of the total samples tested were positive for HIV-1 by CAP/CTM HIV-1 Qualitative assay, whereas 55(37.4%) samples were positive by GenXpert HIV-1 Qualitative assay. There were no invalid results reported in the reference method, whereas 10(6.8%) samples were invalid in the GenXpert HIV-1 Qualitative assay. However, error results were not reported for either method. The diagnostic performance of the GenXpert HIV-1 Qualitative assay was determined by using the CAP/CTM HIV-1 Qualitative assay as a reference method. The sensitivity and specificity of the GeneXpert HIV-1 Qualitative assay was 100% and accuracy of the assay against the reference method was also 100% (95% CI, 97.3-100) (Table 1).

Table 1: Diagnostic performance GenXpert HIV Qualitative testing method compared with the CAP/CTM HIV Qualitative assay

Statistical parameters	Value	95% CI	
		Lower limit	Upper limit
Sensitivity	100%	93.5%	100%
Specificity	100%	95.6%	100%
Kappa value	1.00		
Accuracy	100%	97.3%	100%

Integration and national scale-up of Xpert HIV-1 Qualitative testing to the existing TB diagnostic facilities using GeneXpert: Based on the results of the pilot implementation, the government decided to expand Xpert HIV-1 Qualitative testing to 69 sites in the first phase and to 50 in the second. The sites were chosen based on their TB caseload and available spare capacity for EID testing. Before scaling up, the sites' readiness was assessed, training, job aids, logbooks, and necessary materials were provided; the GeneXpert software version was upgraded to include the Xpert HIV-1 Qualitative assay definition, and defective modules were identified and replaced with functional ones.

In this pilot implementation study, 11 health facilities were included throughout the country. A total of 1,587 samples (982 whole blood and 605 DBS) were collected. The minimum number of samples was collected from Addis Ketema Health Centre, 5(0.3%), and the maximum number was

collected from Shashemene Hospital, 264(16.6 %). Of the total whole blood and DBS samples, 1,216(76.6%) were tested by GenXpert HIV-1 Qualitative testing assay during the pilot compared with 371(23.4%) done using the conventional testing method (CAP/CTM HIV-1 Qualitative test), which is centralized EID testing during the period which was covered by the baseline assessment. HIV-1 was detected in 48/1216 (4%) of the whole blood and DBS samples tested by Xpert and in 25/371(6.7%) of those tested by CAP/CTM HIV-1 Qualitative test.

Regarding TAT, 993(62.6%) samples were tested with the GeneXpert HIV-1 Qualitative assay and the results were delivered within the same day. There were 12 (0.8 %) deaths among all HIV exposed infants in the pre-POC EID pilot implementation phase when tests were done with the conventional method as compared to zero during the pilot implementation phase where GeneXpert was used (Table 2).

Table 2: Characteristics of EID testing for 982 whole blood and 605 DBS samples at different health facilities by both the conventional and GeneXpert methods

Variable		Frequency	Percent
Facilities	Addis Ketema HC	5	0.3
	Ambo Hospital	31	2.0
	Bishoftu Hospital	60	3.8
	Dilchora Hospital	129	8.1
	Gambella Hospital	188	11.8
	Gandi Hospital	32	2.0
	Karamara Hospital	15	0.9
	Maychew Hospital	134	8.4
	Mizan Hospital	171	10.8
	Ras Desta Hospital	9	0.6
	Shashemane Hospital	264	16.6
	St Luke Hospital	168	10.6
	Tirunesh Bejing Hos	11	0.7
	Woldia Hospital	185	11.7
	Zewditu Hospital	185	11.7
Sample type	Whole Blood	982	61.9
	DBS	605	38.1
Number samples tested by each assay	Conventional	371 DBS	23.4
	GenXpert	1216 (982 whole blood, 234 DBS)	76.6
Test Result by both assays	Negative	1514	95.4
	Positive	73	4.6
TAT (Day) for both assays	≤ 1	993	62.6
	>1	594	37.4
Status of the infants during the study period	Alive	1575	99.2
	Dead	12	0.8
	Total	1587	100.0

In this study, there was significant difference between the GeneXpert HIV-1 Qualitative assay and the conventional method (CAP/CTM HIV-1 Qualitative assay) among the facilities, sample type,

TAT and infant HIV status ($p < 0.001$). However, there were no significant differences between the conventional and GenXpert methods among EID results ($P = 0.33$) (Table 3).

Table 3: Chi-square analysis of differences between CAP/CTM and Xpert HIV Qualitative assays

Category	Variables	Methods		Chi square(p-value)
		CAP/CTM (conventional) HIV-1 Qualitative assay N (%)	GenXpert HIV-1 Qualitative assay N (%)	
Sample type	Whole blood	0 (0.0)	982 (80.8)	<0.001
	DBS	371(100.0)	234 (19.2)	
TAT	≤ 1 day	0 (0.0)	993(81.6)	<0.001
	>1 day	371 (100.0)	223(18.4)	
Infant status	Alive	359 (96.8)	1216 (100.0)	<0.001
	Dead	12 (3.2)	0 (0.0)	
EID result	Negative	346 (93.3)	1168 (96.0)	0.33
	Positive	25 (6.7)	48 (4.0)	

In this study, TAT was significantly lower in the Xpert HIV-1 Qualitative assay compared to the conventional one (CAP/CTM HIV-1 Qualitative assay). In the conventional centralized EID testing, TAT was impacted by different factors from sample collection to result back to healthcare workers. As a

consequence, the length of time it took to ship samples to centralised EID testing laboratories was the most significant predictor of long TAT, whereas the length of time it took for healthcare professionals to get test results was the least significant predictor (Figure 2).

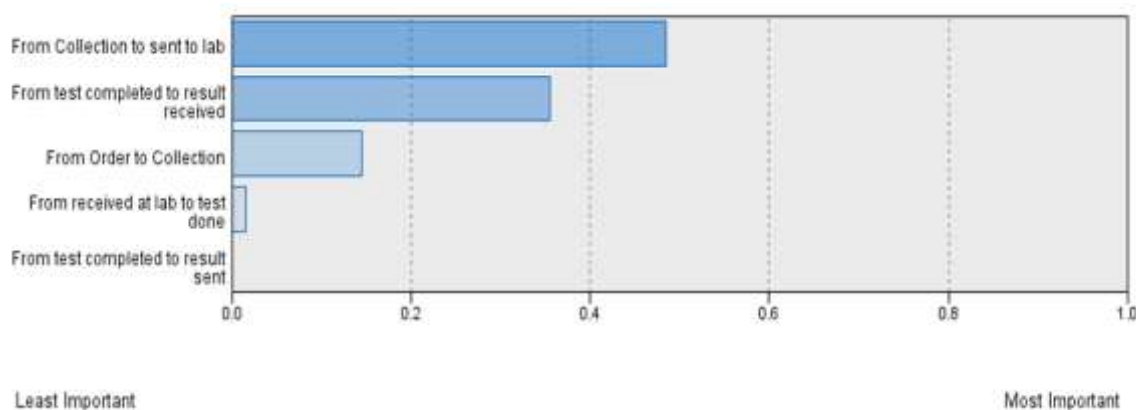


Figure 2: Predictors importance of TAT in the specimen referral networks

Discussion

The utilization rate of the existing conventional EID testing capacity in Ethiopia is low partly due to logistic challenges for transportation of specimens to centralized laboratories. Moreover, prolonged TAT observed in the delivery of the results to the sites hinders early initiation of treatment. This study was intended to evaluate the performance of POC GeneXpert HIV-1 Qualitative assay (first phase) and then assess the implementation of the assay as a pilot in selected health facilities in Ethiopia (second phase).

In Ethiopia, Xpert HIV-1 Qualitative (POC EID test) on the GeneXpert platforms was introduced at eleven government health facilities (Eight for standalone EID test and three integrated with TB tests on the same platform), for testing at six-weeks after birth. The integration of both tests was performed based on the case load of TB and/or the current new test (Xpert HIV 1 Qualitative) at the selected health facility. Hence, this was successful, and the service was well run throughout the implementation period. The first phase of this study was performing verification testing using previously tested DBS samples with the conventional testing method (CAP/CTM HIV-1 Qualitative test) and had known results.

The pre-implementation verification analysis showed that, the performance of the Xpert HIV Qualitative assay had perfect agreement (100% sensitivity and specificity, K value=1.00) compared with the CAP/CTM HIV-1 Qualitative assay version 2.0 in DBS samples which were collected from exposed infants. Similar results were reported from Tanzania (100% sensitivity and specificity) (Sabi et

al. 2017), India (97% sensitivity and >97% specificity) (Kulkarni et al. 2017) and Kenya (99.23 % sensitivity, 98.91% specificity and 99% accuracy) (Bwana et al. 2019). On the contrary, in a study conducted in South Africa, the specificity of Xpert HIV Qualitative assay was comparatively lower (specificity was 85.7%). The reason for this difference might be sample size, study design and setting (Mukendi et al. 2021). Based on the above findings, the diagnostic performance of POC Xpert HIV Qualitative assay had comparative performance compared to the conventional CAP/CTM HIV-1 Qualitative assay version 2.0. However, in this study, 10 (6.8%) samples were invalid/error in the Xpert HIV-1 Qualitative assay on the first run of detection. Similar finding was reported from Kenya (6.44% error/invalid rate) (Bwana et al., 2019). On the other hand, higher error/invalid rate was recorded in South Africa in two studies, (9.6% error rate)(Spooner et al. 2019) and 14.1% error/invalid rate (Kufa et al. 2020). All these reports, including our findings indicated that, the error/ invalid rate was higher than the WHO prequalification report (2.2% invalid rate). Error/ invalid results of a sample was highly related to sample quality, sample preparation and sample volume used for EID testing as well as extra training and support needs to the clinical staff (Agutu et al. 2019).

After successful verification, routine testing was commenced at the end of May 2017 to April 2018 and out of a total of 1216 infants tested using Xpert HIV-1 Qualitative assay, HIV-1 was detected in 48 (4 %). In the present study, the positivity rate of HEI was higher than study finding from South Africa (1.4%) (Technau et al. 2017) and lower than in

Democratic Republic Congo (16.5%) (Rubio-Garrido et al. 2019).

In this study, testing efficiency by comparing outcomes of different indicators between the Xpert HIV-1 Qualitative point of care testing and conventional CAP/CTM testing was also assessed. The most significant indicator that could affect the quality of care was found to be TAT. TAT was significantly lower in Xpert POC testing as compared to the conventional testing method, although 223(18.4%) of Xpert HIV Qualitative results TAT was greater than one day due to some of near POC health facilities referred DBS sample to the testing site. However, all samples tested centrally using conventional CAP/CTM platforms had a TAT of more than one day. The delay occurs at different points in the path throughout the specimen referral network. In this path there are different concerned bodies including, sample collection sites (mainly delayed at PMTCT clinics), referring laboratory (delayed communication with postal courier) and the postal courier (delay during transportation). The TAT predictor analysis showed that, almost 50% of the delay occurred from sample collection at the patient care setting until it reached the central testing laboratory. A long TAT from specimen collection to result receipt at the clinic, and an even longer time for results returned to mother/caregivers contributes to high loss-to-follow-up. Many other studies also showed that, Xpert HIV Qualitative testing assay for EID greatly improved the testing efficiency, TAT of result return to patients and documentation (Spooner et al. 2019; Bwana et al. 2019; Mohamed et al. 2020). Therefore, introducing POC significantly improves key EID outcomes, such as short TAT to ART initiation (Dunning et al. 2015).

Limitations of the study

As the study depended on routinely collected data from healthcare settings, some data quality errors were expected. However, there were very few missing data, and extensive and frequent data quality checks minimized the potential for errors.

Conclusion

This pilot study has demonstrated that the GeneXpert POC EID assay has a high sensitivity and specificity as compared to the reference method. The pilot also showed that Xpert HIV-1 Qualitative assay improved access to same day testing of HEI and facilitated putting them on ART as soon as possible, if they tested positive. The practicability of this assay makes it suitable for health facilities far from DNA PCR testing labs with high case load. Therefore, Xpert HIV-1 Qualitative assay offers significantly improved care for HEI and Ethiopia should consider the strategic addition of POC testing to the diagnostic algorithms and health facilities to improve early detection and treatment initiation to those who test positive. Integrating EID on TB

testing using GeneXpert devices is also feasible and cost-effective.

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