ORIGINAL ARTICLE

Retrospective Study

Active tracking of rejected dried blood samples in a large program in Nigeria

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Institutional review board statement: The study was approved by the Institutional Review Board and Ethics Committee of the Institute of Human Virology, Nigeria and the National Human Research and Ethics Committee (NHREC Approval#NHREC /01/01/2007-15/08/2015). No patient identifying information was retained. Data analysis was unlinked and anonymous. With delinking of patient identifiers and confidentiality safeguards, the benefits of improved health care quality outweigh the minimal risks.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used secondary de-identified/anonymous clinical data that were obtained after each patient agreed to be enrolled in our treatment program.

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Abstract

AIM: To study the impact of rejection at different levels of health care by retrospectively reviewing records of dried blood spot samples received at the molecular laboratory for human immunodeficiency virus (HIV) early infant diagnosis (EID) between January 2008 and December 2012.

METHODS: The specimen rejection rate, reasons for rejection and the impact of rejection at different levels of health care was examined. The extracted data were cleaned and checked for consistency and then de-duplicated using the unique patient and clinic identifiers. The cleaned data were ciphered and exported to SPSS version 19 (SPSS 2010 IBM Corp, New York, United States) for statistical analyses.

RESULTS: Sample rejection rate of 2.4% (n = 786/32552) and repeat rate of 8.8% (n = 69/786) were established. The mean age of infants presenting for first HIV molecular test among accepted valid samples was 17.83 wk (95%CI: 17.65-18.01) *vs* 20.30 wk (95%CI: 16.53-24.06) for repeated samples. HIV infection rate was 9.8% *vs* 15.9% for accepted and repeated samples. Compared to tertiary healthcare clinics, secondary and primary clinics had two-fold and three-fold higher likelihood of sample rejection, respectively (P < 0.05). We observed a significant increase in sample rejection rate with increasing number of EID clinics (r = 0.893, P = 0.041). The major reasons for rejection were improper sample collection (26.3%), improper labeling (16.4%) and insufficient blood (14.8%).

CONCLUSION: Programs should monitor pre-analytical variables and incorporate continuous quality improvement interventions to reduce errors associated with sample rejection and improve patient retention.

Key words: Human immunodeficiency virus; Prevention of mother-to-child transmission; Early infant diagnosis; Dried blood spot; Pre-analytical error; Sample rejection

Core tip: For early infant diagnosis of human immuno-deficiency virus, the samples of choice are dried blood spots (DBS). DBS samples are received from over 100 health care centers at the Asokoro Laboratory Training Centre. When DBS arrives the laboratory, a technician receives the samples as well as all accompanying laboratory request forms and all relevant documentation. All routinely collected DBS samples are physically examined for quality and acceptability for molecular testing upon reception at the laboratory. Only samples that meet the laboratory acceptance criteria are usually tested. Samples which fail to meet the acceptance criteria are registered in the sample rejection logbook without being tested. All DBS samples accepted as fit-for-testing are electronically registered into the laboratory information management system (LIMS). The use of the LIMS reduces instances of transcriptional errors. DBS samples are processed using real-time PCR technology on the Cobas Taqman and Cobas ampliprep equipment. DBS samples are cut, eluted into solution, and then placed in the equipment where DNA extraction, amplification and detection is automatically carried out. Once results are ready, they are validated by the laboratory scientist for accuracy and completeness. If assay is judged to be a valid run, the assay is accepted with a click of a computer button.

INTRODUCTION

The recognition of prevention of mother-to-child trans-mission (PMTCT) as an essential tool for combating the human immunodeficiency virus (HIV) epidemic has led to its institution by the World Health Organization (WHO) as a global health agenda[1]. PMTCT programs can reduce the risk of MTCT to less than 2%, and is today the most efficacious tool for preventing pediatric HIV infection globally[2-5]. PMTCT programs have witnessed appreciable success in Nigeria with documented MTCT rates ranging from 1.3%-4.8% in mother-baby pairs who received antiretroviral therapy (ART), compared to MTCT rates ranging from 39.8%-68.0% where no intervention was administered[6-9]. Nevertheless, MTCT is still a critical challenge of the HIV/AIDS pandemic in resource limited settings (RLS)[10-13]. According to UNGASS country reports, only 30.1% of HIV positive pregnant women in Nigeria received ARV drugs to prevent MTCT in 2013, which resulted in MTCT rates as high as 27.3%. And in the same year, only 3.9% of exposed infants received a PCR diagnostic test within two months of birth[14]. This low level of diagnosis among HIV-exposed infants falls below the national target of ensuring that at least 90% of all HIV exposed infants have access to early diagnosis services by 2015[5]. Especially with an estimated 52125 to 104250 infants at risk of being infected with HIV without intervention[15].

Early testing of exposed infants from 4 to 6 wk of birth is recommended by the WHO to insure timely diagnosis and treatment of HIV positive children[1,16]. Without intervention HIV causes a 20% mortality rate in infected infants in RLS by 3 mo of age, which increases to an estimated 48% and 52% before ages one and two, respectively[16,17]. Despite this, the average age of initiation of ART in pediatric HIV/AIDS patients in RLS remains high[17,18], and health-care systems often fail to meet the national demands for care[1]. In 2012 only 12% of children eligible for ART received treatment in Nigeria[19]. Reports also show high rates of loss to follow-up (LTFU) of infants throughout the PMTCT cascade in RLS, with an over 30% rate of LTFU by 3 mo and more than 70% by 6 mo of birth[20-23]. It is estimated that only 0.5% to 52.8% of infants eligible for early infant diagnosis (EID) testing in RLS complete the care cascade and eventually access treatment[22]. Therefore, strategies for improving patient retention should be a critical focus of PMTCT programs with respect to the UNAIDS 90-90-90

targets[5]. A review of the PMTCT cascade is essential to identify gaps towards achieving the goals of PMTCT services[24]. Careful consideration of the role of laboratory in ensuring early diagnosis and universal access to pediatric ARVs is also vital to ensure the widest possible coverage of PMTCT services[25].

EID is a vital intervention which allows countries to provide essential health services for all children and to continue to make progress in keeping children alive and healthy. Standard HIV antibody testing - as is done with adults and older children - cannot identify infected infants in their first year of life, as it also detects maternal HIV antibodies that are transferred to the baby during pregnancy (and subsequently decline slowly within the first year of life)[8,9]. More demanding testing methods that rely on detecting HIV-1, or virological tests are required for diagnosing infants[19]. HIV DNA PCR test is the most widely used initial assay for EID in industrialized countries[1]. Early HIV virological detection test at or after 6 wk of age for all HIV-exposed children identifies most children infected before, during and immediately after delivery[6-9].

The guideline for early infant diagnosis in Nigeria provides that all HIV exposed infants have a first HIV diagnostic test at 6 wk of age, a follow-up test at 6 wk after cessation of breastfeeding and a confirmatory HIV test at 18 mo[26-28]. Pre-analytical errors contribute an estimated 60%-70% of all mistakes in laboratory diagnostics and can render dried blood spots (DBS) untestable, leading to specimen rejection with a resultant negative impact on patients[29-31]. Common pre-analytical errors associated with DBS rejection include: Labeling errors, sample damage, missing or inconsistent data, and insufficient volume[32-35]. High risk for rapid disease progression and death necessitates the need for early identification and treatment of HIV positive infants[36]. The goal of the present study was to investigate the DBS sample rejection rate attributable to pre-analytical errors and its effect on patient care in the PMTCT cascade at the tertiary, secondary and primary levels of healthcare service delivery in Nigeria and provide strategies to reduce effectively to nil rejection at all levels of healthcare service delivery in Nigeria.

MATERIALS AND METHODS

Study setting and design
This is a cross-sectional descriptive study conducted among HIV-exposed babies from 150 health facilities using prospectively collected data from the molecular diagnostics laboratory of Institute of Human Virology, Nigeria (IHVN). The IHVN is a not-for-profit organization established in 2004 to scale up the US PEPFAR program in Nigeria and conduct research and training towards improving quality and promoting evidence based health system strengthening[37]. The IHVN currently has 10 out of the 26 molecular diagnostic laboratories across the six geopolitical regions of the country.

Laboratory data collected over a 5-year period from

January 8, 2008 to December 19, 2012 were retrieved from the laboratory's information management Microsoft Excel database. The dataset included the following variables: (1) Date of sample collection; (2) Patient's hospital number; (3) Laboratory number; (4) Date specimen was received at the laboratory; (5) Specimen type; (6) Reason for DNA PCR test (first test for healthy exposed baby, first test for sick baby, follow-up test to confirm first test, follow-up test after cessation of breastfeeding); (7) Specimen suitability for analysis (accepted or rejected); and (8) Reasons for sample rejection and other demographic information. The demographic information included: (1) Patient's age; (2) Patient's sex; (3) PMTCT intervention administered to mother; (4) PMTCT intervention administered to patient (exposed infant); (5) Breastfeeding status; and (6) DBS collection clinic. The dataset included information on samples received at the molecular diagnostics laboratory from 150 healthcare centers including tertiary (n = 9; 6%), secondary (n = 101; 67%) and primary (n = 40, 27%) healthcare centers within the Northern region of Nigeria.

Sample history
All routinely collected DBS samples were examined for quality and acceptability for molecular testing upon reception at the laboratory. Valid specimens were accessioned and registered into the laboratory information management register and Microsoft Excel template. Only samples that met the laboratory acceptance criteria were tested. Samples which failed to meet the acceptance criteria were registered in the sample rejection log without being tested. The laboratory records for accepted (valid) and rejected samples were merged using the patient's hospital number and collection healthcare clinic identifiers.

Reasons for sample rejection
Reasons for sample rejection included: Sample quantity insufficient for testing; Sample not properly labeled with patient's name, patient's hospital number and the name of the collection clinic; Improperly collected sample. This includes all specimens which appeared diluted, had alcohol halo or serum ring around it and specimen which appeared abraded, over-saturated, clotted, caked or layered; Sample that appeared discolored or contaminated; Sample not properly packaged separately to avoid cross-contamination; Sample not allowed to dry completely before packaging and mailing; Sample for babies younger than 6 wk or older than 18 mo of age; and sample received without a patient/test request form.

Study variables
The sample rejection rate was the primary outcome variable in this study. The type and frequency of pre-analytical errors associated with sample rejection and the repeat rate for rejected samples were also determined relative to the type of healthcare center where the sample was collected. We also evaluated the HIV-1 positivity rate and the mean age among infants presenting for HIV-1

Table 1 Dried blood spot sample rejection rate by year

|		Year					Total
		2008	**2009**	**2010**	**2011**	**2012**	
Rejected?	No	2117 (6.5%)	5186 (15.9%)	6634 (20.4%)	8759 (26.9%)	9070 (27.9%)	31766 (97.6%)
Count (%)	Yes	2 (0.1%)	62 (1.2%)	223 (3.3%)	166 (1.9%)	333 (3.5%)	786 (2.4%)
Total		2119 (6.5%)	5248 (16.1%)	6857 (21.1%)	8925 (27.4%)	9403 (28.9%)	32552 (100%)
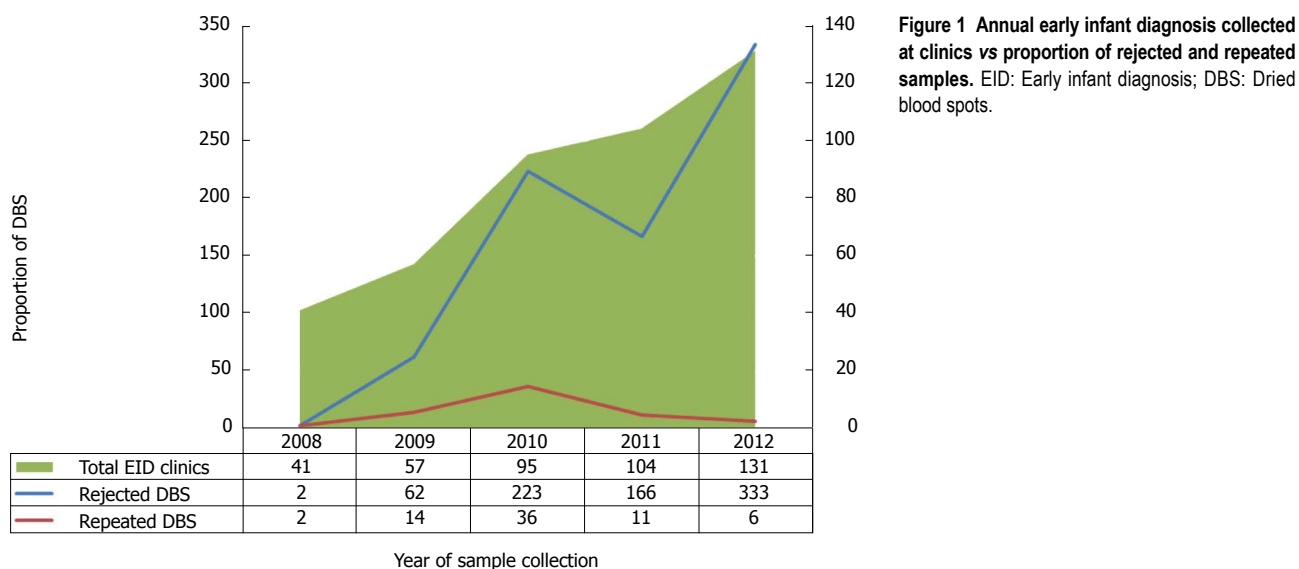
Figure 1 Annual early infant diagnosis collected at clinics *vs* proportion of rejected and repeated samples. EID: Early infant diagnosis; DBS: Dried blood spots.

	2008	2009	2010	2011	2012
Total EID clinics	41	57	95	104	131
Rejected DBS	2	62	223	166	333
Repeated DBS	2	14	36	11	6

DNA PCR test for accepted and repeated samples.

Statistical analysis

The extracted data were cleaned, checked for consistency and then de-duplicated using the unique patient and clinic identifiers. The cleaned data were ciphered and exported to SPSS version 19 (SPSS 2010 IBM Corp, New York, United States) for statistical analyses. We used descriptive statistics to establish the DBS sample rejection rate and the reasons for rejection; and to determine the mean age of infants presenting for first HIV-1 DNA PCR test and for a follow-up test. Logistic regression analysis was used to test the difference in sample rejection rate between the different types of healthcare centers providing care. Furthermore, we used Pearson correlation coefficients (r) to test the association between the annual sample rejection rate and the number of clinics providing EID services. A P-value < 0.05 was considered statistically significant. The statistical review of the study was performed by a biomedical statistician.

RESULTS

After the data cleaning process, 32552 sample data from laboratory records over the five-year study period were included in the analysis. A total of 6322/32552 (19.4%) samples were sent from tertiary health clinics, 24777/32552 (76.1%) from secondary health clinics, and 1453/32552 (4.5%) from primary health clinics. Based on the laboratory's sample rejection criteria,

786/32552 (2.4%) samples were found to have been rejected. Only 8.8% of those rejected samples were repeated. Primary healthcare clinics had the highest rejection rate of 4.0%, while secondary and tertiary healthcare clinics had rejection rates of 2.6% and 1.3%, respectively. Secondary healthcare clinics had a twice greater probability (OR = 1.955; 95%CI: 1.557-2.455) and primary healthcare clinics had more than 3 times higher probability (OR = 3.051; 95%CI: 2.174-4.281) of DBS sample rejection when compared to tertiary health care clinics (P < 0.05). The repeat rates were 1.7%, 8.7%, and 14.1% for primary, secondary and tertiary healthcare centers, respectively.

As shown in Table 1, the cumulative sample rejection rate increased from 0.1% in 2008 to 3.5% in 2012, while the repeat rate of rejected samples decreased across the study period (Figure 1) from 2/2 (100%) to 6/333 (1.8%). The sample rejection rate also increased with increasing number of EID DBS collection clinics (Figure 2) in the PMTCT program (r = 0.893, P = 0.041).

We observed a high mean age of 17.83 wk (SD = 15.29; 95%CI: 17.65-18.01) for infants presenting for first EID test in the program. A higher mean age of 20.30 wk (SD = 14.31; 95%CI: 16.53-24.06) was recorded for repeated samples among infants presenting for a first EID test. The mean age of infants for all repeated samples including patients presenting for first test and follow-up test was 22.32 wk (SD = 15.49; 95%CI: 18.60-26.05) *vs* 19.95 wk (SD = 16.43; 95%CI: 19.77-20.14) among samples that were accepted at first collection. Additionally, the mean

Table 2 Reasons for sample rejection by type of healthcare care center (*n* = 786)

Reason for rejection count (%)	Tertiary	Secondary	Primary	Total
No DBS card	5 (0.6)	76 (9.7)	4 (0.5)	85 (10.8)
Insufficient quantity of sample	15 (1.9)	72 (9.2)	29 (3.7)	116 (14.8)
No request form	6 (0.8)	39 (5.0)	0 (0.0)	45 (5.7)
Improper collection	25 (3.2)	171 (21.9)	11 (1.4)	207 (26.3)
Baby over age (≥ 18 mo)	6 (0.8)	58 (7.4)	0 (0.0)	64 (8.1)
Improper labeling	17 (2.2)	110 (14.0)	2 (0.3)	129 (16.4)
Improper packaging	4 (0.5)	53 (6.7)	8 (1.0)	65 (8.3)
Contaminated sample	0 (0.0)	5 (0.6)	0 (0.0)	5 (0.6)
Baby under age (< 6 wk)	2 (0.3)	19 (2.4)	2 (0.3)	23 (2.9)
Reason unknown	6 (0.8)	40 (5.1)	1 (0.1)	47 (6.0)
Total	86 (10.9)	643 (81.8)	57 (7.3)	786 (100.0)

DBS: Dried blood spot.

Figure 2 Annual total early infant diagnosis collected at clinics *vs* sample rejection rate. EID: Early infant diagnosis; DBS: Dried blood spots.

Year of sample collection	2008	2009	2010	2011	2012
EID DBS collection clinics	41	57	95	104	131
DBS rejected rate	0.1	1.2	3.3	1.9	3.5
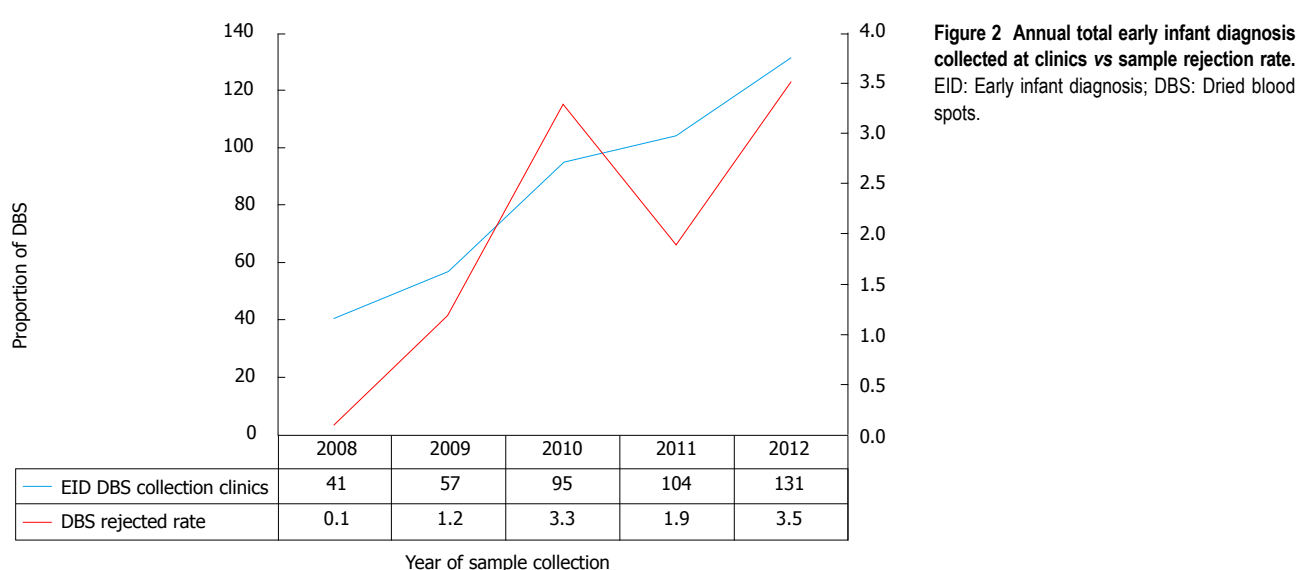
age was 33.02 wk (SD = 17.70; 95%CI: 21.13-44.91) for those presenting for a follow-up test among repeated samples *vs* 35.55 wk (SD = 16.09; 95%CI: 35.03-36.08) for accepted samples. We established a cumulative positive rate of 9.8% for all accepted samples routinely tested over the 5-year period while the positive rate for repeated samples was 15.9%.

The average turn-around time from sample collection at the health facility to receipt of sample at the laboratory was 3.82 wk ± 3.63 (95%CI: 3.69-3.95). Overall, the most frequently occurring errors associated with sample rejection were improper sample collection (*n* = 207/786; 26.3%), improper labeling (*n* = 129/786; 16.4%) and insufficient blood (*n* = 116/786; 14.8%). Other reasons for rejection included improper packaging, no sample sent, no test request form sent, baby over-age (> 18 mo), baby under-age (< 6 wk) and contaminated sample (Table 2).

DISCUSSION

The mean age of infants at first HIV DNA PCR test in this study is far beyond the recommended age of 4-6 wk for EID testing[1]. Without treatment, HIV related mortality in infected infants peaks at 8 to 12 wk[38]. Delay in presentation for EID averts the opportunities to administer ARV and reduce MTCT[6,7,39], thereby permitting the emergence of more severe clinical manifestation of HIV infection in pediatric patients[40]. Strategies that enhance awareness of PMTCT and EID services, promote partner involvement, provide economic incentives and offer close follow-up to HIV positive women during pregnancy and after delivery have been shown to be effective[41,42]. Active tracking of HIV positive mothers using support groups and mobile applications have also been shown to increase uptake of services and retention of the mother-baby pair in PMTCT programs[41,43,44].

Establishing an accurate link between rejected samples and the impact on clinical outcome is difficult[32]. However, the observed high rejection and low repeat rates in addition to the higher mean age of infants at the time of specimen recollection in this study suggest that sample rejection further delays HIV diagnosis in infants while emphasizing the importance of standardization and monitoring of pre-analytical variables[30]. Our study agrees with other investigations where pre-analytical

errors are implicated in delayed diagnosis of infant HIV[33-35,45]. Other adverse patient outcomes due to sample rejection include demand for patient revisits for specimen recollection, discomfort to the patient, test abandonment or LTFU and time lost in waiting for results with the accompanying cost implications associated with multiple clinic visits[22,46,47]. The extended delay in results may also have contributed to the high attrition and low repeat rates among rejected samples.

Due to the importance of accurate and timely diagnosis in the care and treatment of HIV positive children and the increased risk for postnatal transmission, morbidity and early mortality in untreated HIV[6,7,48], greater attention to sample quality, clear guidelines on the responsibility and protocols for sample collection, error reporting and initiating patient follow-up for timely specimen recollection should be established. The high turn-around time of 3.82 wk ± 3.63 wk from sample collection to receipt at the testing laboratory also suggests the need for improved systems for rapid sample transportation[49]. Lack of standardized protocols for laboratory processes including sample collection, specimen acquisition, management and storage contributes up to 93% of errors in diagnostics[50]. Implementing standardized protocols for reporting and managing non-conformance events can also improve service performance[46,47].

The majority of the samples in our study were rejected due to improper collection, a factor attributable to personnel error and is seen to be highest in secondary health clinics where the number of patients presenting for EID testing is highest. A recent study reported that staff sensitization on patient preparation, test request forms, and sample management significantly reduced pre-analytical error rate from 19.07% to 6.76%[47]. Thus, programs should intensify monitoring of pre-analytical staff, processes and performance towards improving sample quality[25,30,46,51].

Significant correlations between the annual number of DBS sample collection clinics and the annual sample rejection rate also suggests that an increasing number of EID clinics can put a strain on the program. Increased focus on site-based EID training and mentoring activities through 2011 is thought to be responsible for the decline in DBS sample rejection observed in that year. The shift to accelerated scale-up and decentralization of PMTCT services to primary health clinics where Community Health Extension Workers constitute a greater percentage of the workforce may have contributed to the peak in sample rejection recorded in succeeding year, 2012[52-54]. This may also explain the higher relative risk of sample rejection in primary health clinics.

Lapses in control, monitoring and supervision in the pre-analytical phase of clinical laboratory services and sample collection by non-laboratory personnel have been implicated as red flags for error propagation[55,56].

In the present study the infection rate among accepted samples and repeated samples (9.8% *vs* 15.9%) is in agreement with previous findings that LTFU can lead to low levels of detection of HIV infection in infants and missed opportunities for care[22,57]. Active patient tracking systems that use social workers to track patients have been applied in Kenya to reduce LTFU among HIV, PMTCT and tuberculosis patients from 21% to 15%[43]. In other studies, peer-based strategies that engage expert and or mentor-mothers in educating and motivating HIV positive mothers to access PMTCT services using their own experience, have been instrumental in improving retention of mother-baby pair in care[58,59]. Interventions should therefore seek to educate mothers and guardians on the grave importance of early diagnosis in pediatric HIV.

Although Quality Management System (QMS) is still seeing little application in Nigeria, an effective QMS is critical to the success of the laboratory testing networks[28,29]. Recent studies report that application of Quality Improvement tools such as Rapid Results Initiative and Continuous Quality Improvement interventions that seek to identify and correct system defects can significantly reduce sample rejection and increase patient retention in PMTCT programs in the similar setting[41,44].

Limitations

The current study is a retrospective analysis of laboratory records which are often incomplete as evidenced by the proportion of rejected samples with unknown reasons for rejection. This can introduce misclassification or information bias. Also it is often difficult to accurately interpret retrospective data and the quality of data collected over time. We did not investigate the reason for requesting an HIV test for samples collected for a follow-up test among repeated samples. This then does not reflect the actual mean age of infants presenting for a follow-up test among rejected samples as we could not determine if the tests were follow-up due to sample repeat or true follow-up tests.

Given the small size of the rejected samples compared to the total number of routinely collected samples, we did not test the statistical significance of the comparative analysis between these groups. Additionally, due to incomplete documentation we could not determine the mean age of infants presenting for HIV-1 DNA PCR test for the rejected samples.

In conclusion, the study demonstrates that DBS sample rejection can further delay HIV-1 EID testing, contributes to LTFU and adversely impacts program and patient outcomes at various levels of healthcare. An integrated multidisciplinary approach which engages social support groups, health personnel, quality improvement interventions as well as electronic and mobile communication tools is needed to improve uptake of PMTCT services and the overall health outcome of HIV positive mothers and their infants. Intensified training and monitoring of personnel, quality policies for sample collection and patient follow-up should be integrated into the scale-up agenda to prevent sample rejection

and promote recollection when errors occur. Other considerations should include continuous counseling and active tracking of mothers and care givers to improve patient retention and achieve the goals of PMTCT programs.

COMMENTS

Background
Studies reveal that antiretroviral therapy can reduce rate of mother-to-child transmission of human immunodeficiency virus (HIV) to less than 2%. However, over 30% of HIV-exposed infants in resource limited settings are lost to follow-up by 3 mo of life and only 0.5%-52.8% of these infants are successfully enrolled into care and treatment.

Research frontiers
Eight countries (Nigeria, South Africa, India, Mozambique, Tanzania, Zimbabwe, Uganda and Kenya) accounted for 58% of the global acquired immunodeficiency syndrome (AIDS)-related deaths in 2013. Without antiretroviral preventive interventions for prevention of mother-to-child (PMTC), the risk of perinatal HIV transmission has varied between 15% and 45%, depending on maternal risk factors and whether breastfeeding is practiced. Nigeria has the highest number of children contracting the HIV, in the world (UNAIDS 2012). Early testing of exposed infants from 4 to 6 wk of birth is recommended by the World Health Organization to insure timely diagnosis and treatment of HIV positive children. An investigation of gaps in the PMTC transmission (PMTCT) cascade is important to identify improvement areas for optimizing linkage of HIV/AIDS infants into care and treatment.

Innovations and breakthroughs
An investigation of gaps in the PMTCT cascade is important to identify improvement areas for optimizing linkage of HIV/AIDS infants into care and treatment. The use of SMS printers and laboratory information system are major innovations that have been shown to reduce TAT and enhance tracking of rejected dried blood spot samples.

Applications
The shift to accelerated scale-up and decentralization of PMTCT services to primary health clinics where Community Health Extension Workers constitute a greater percentage of the workforce may have contributed to the peak in sample rejection recorded. An integrated multidisciplinary approach which engages social support groups, health personnel, quality improvement interventions as well as electronic and mobile communication tools is needed to improve uptake of PMTCT services and the overall health outcome of HIV positive mothers and their infants. Intensified training and monitoring of personnel, quality policies for sample collection and patient follow-up should be integrated into the scale-up agenda to prevent sample rejection and promote recollection when errors occur.

Terminology
EID: Early infant diagnosis; PMTCT: Prevention of mother-to-child transmission; PCR: Polymerase chain reaction.

Peer-review
This work by Inalegwu *et al*, addresses an important problem of enhanced tracking of rejected dried blood spot samples, which dramatically affects the PMTCT of HIV. The paper is well written, and the data are convincing since they are analyzed with appropriate statistical tools.

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