Early Infant Diagnosis Specimen Rejection Rate at Mutare Provincial Hospital Laboratory, Zimbabwe
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ABSTRACT

Introduction: According to the World Health Organisation (WHO), all HIV exposed infants should be tested by Dried Blood Spots (DBS)-Polymerase Chain Reaction (PCR) before or at 6 weeks of age, in the so-called early infant diagnosis (EID) programme. This is because there have been prior reports of high morbidity and mortality in HIV-infected infants and children due to inadequate care and treatment in many developing countries, mostly due to unknown HIV status. EID testing is a crucial step to facilitate early access to antiretroviral treatment (ART). In order to inform clinicians, policy makers and the public, we carried out a study to determine specimen rejection rate and the reasons for specimen rejection by retrospectively reviewing records of Dried Blood Spot (DBS) samples received at Mutare Provincial Hospital Molecular Diagnostics Laboratory for HIV EID in Zimbabwe.

Aim of Study: To determine the specimen rejection rate and reasons for sample rejection at the EID Laboratory at Mutare Provincial Hospital as a way to ensure continuous availability of good quality diagnostic systems for HIV infected women, their infants and families.

Methods: This was a cross-sectional retrospective study conducted on data for samples submitted to Mutare Provincial Hospital Molecular Diagnostics Laboratory. The data was collected over a 1-year period from January 2015 to December 2015 and was retrieved from the laboratory’s information database in January 2016. The records included date of specimen collection; date specimen was received at the laboratory and reasons for specimen rejection.

Results: The specimen rejection rate was 10.7%. The reasons for rejection included improper specimen collection (24.8%), no specimen received (12.6%), multiple specimens packed in one sample pack (17.2%), specimens and forms not labelled correctly (8.5%), specimens tested that requested for a new specimen due to machine error hence rejected during testing (16.6%), specimens tested but failed (8.2%) and specimens tested with an indeterminate result (12.2%).

Conclusion: The study demonstrates that DBS specimen rejection in our setting (10%) is far higher than the 1-3% reported by the Clinton Health Access Initiatives, a global organisation spearheading research on HIV diagnostic tests in low and middle income countries. Rejection was mainly due to human error (83.4%) hence this necessitates intensified training and monitoring of local personnel and integrating quality policies for specimen collection and processing to prevent specimen rejection. Other considerations could include continuous counselling of clinicians and medical laboratory scientists in order to improve sample quality and specimen handling as a way to achieve the goals of the national EID program.
INTRODUCTION

Zimbabwe is one of the countries in sub-Saharan Africa worst affected by the HIV-epidemic, with a generalised epidemic and an estimated adult HIV prevalence of 15% according to the 2010/2011 Zimbabwe Data Health Survey (ZDHS). The total population of adults and children living with HIV is estimated at 1,550,250 [1, 2]. The country has however registered a gradual decline in HIV prevalence among pregnant women attending antenatal clinic (ANC). According to the ANC serosentinel survey the HIV sero-prevalence among pregnant women decreased from 12% in 2010 and 2011, to 10% in 2012. Though ANC sero-prevalence is declining, in reality the prevalence is still unacceptably high [1, 3].

About 15,000 new paediatric HIV infections were reported annually, in Zimbabwe, as of 2009 and of these new HIV infections, 90% were from mother to child transmission (MTCT) of HIV which is eminently preventable [3]. In developed countries, cases of new HIV infections in children have been virtually eliminated, and MTCT rates are currently as low as <2%, through the use of interventions that include antiretroviral (ARV) drugs for the mother’s own health and for Prevention of Mother to Child Transmission (PMTCT), safe delivery practices and replacement feeding [3]. The Zimbabwe Ministry of Health and Child Care (MoHCC) responded to the high MTCT rates with a strong commitment to scaling up PMTCT services to achieve the targets set by the Global Plan to eliminate paediatric HIV by 2015. Specifically MoHCC aimed to reduce the number of new HIV infections in children by 90% and reduce MTCT to less than 5% [4]. The Zimbabwe PMTCT program in its current Option B+ plan has adopted the MoHCC vision to reduce the mother to child transmission of HIV to less than 5% [1]. Some PMTCT targets have been met and efforts continue being made to sustain PMTCT coverage to over 90% and to reduce MTCT to below 5% by 2020 [2].

While early EID is generally available in all target facilities in Zimbabwe, delays in returning results to facilities and to the mothers and overall coverage of EID services remains suboptimal at around 58% [5]. The Zimbabwe MoHCC recently conducted individual district specific bottle neck analyses and reported that the main bottle necks include consistently low EID coverage, high sample rejection rates and long turn-around times to get EID results amongst many others [5]. Delays in confirmatory diagnosis have been attributed to the cause of high morbidity and mortality among HIV positive babies hence early infant diagnosis (EID) of HIV, ideally at 6 weeks of age, for babies born to HIV positive mothers is critical in enabling effectiveness of program interventions in Antenatal Clinics (ANC), during labour and delivery [6, 7]. EID helps to confirm diagnosis of the baby to allow early treatment provision which averts morbidity and mortality attributed to HIV [8, 9]. The World Health Organisation (WHO) recommends that the HIV exposure status of infants be determined at the first contact with the health system, ideally before 6 weeks of age [9].

All sites providing PMTCT and follow-up services for HIV exposed infants must be able to collect samples for production of dried blood spots (DBS) for polymerase chain reaction (PCR) HIV DNA testing, the so called early infant diagnosis (EID), even when specimens are processed elsewhere [10].

EID is a crucial step to facilitate access to antiretroviral therapy (ART), to improve infants’ survival and to augment the benefit of PMTCT programmes. Early initiation of ART has been associated with better treatment outcomes and increased survival of children born to HIV infected mothers [11, 12]. Globally, only 15% of HIV exposed babies, access EID within 45 days of birth. Studies showed that about 20% of HIV positive infants die before 6 months and 35% to 40% die before 12 months [13]. Hence timely EID of HIV in infants and children cannot be over-emphasised: it enables the early identification of babies who may require HIV treatment and care; those who are HIV-exposed and assists in the effective use of essential resources by targeting ART on children who need treatment; improves the psychosocial well-being of families and children, reducing potential stigma, discrimination and psychological distress for HIV-uninfected children and increasing the chances of adoption for orphans;
facilitates life-planning for parents and/or children who have HIV [9, 11, 12, 13]. Unfortunately, diagnosis of HIV infected infants often occurs too late to allow early initiation of ART in many African countries [10]. The use of DBS test is one of the most effective strategies for increased uptake of EID, as it enables specimen collection and preparation in rural sites that do not have access to health facilities [4]. Studies have suggested significant loss at each step in the EID cascade which has important impacts on the ability to test and treat. The Clinton Health Access Initiative (CHAI) reports a specimen rejection rate for EID samples of between 1 and 3% amongst 26 countries. CHAI is an organisation that drives research meant to forecast global demand for HIV testing in low and middle income countries from 2015 to 2020. The technical group comprises global bodies such as the WHO, Centers for Disease Control and Prevention (CDC), CHAI, Partnership for Supply Chain Management, the Global Fund to Fight AIDS, TB and Malaria, UNAIDS and UNICEF amongst others [14, 15]. The EID dried blood spot (DBS) sample collection programme in Zimbabwe is currently being implemented in 1485 health facilities, herein referred to as clinics. In each of the clinics, infants are bled by nurses and the DBS samples are sent to the three diagnosing laboratories - Mpilo Hospital Laboratory in Bulawayo, Harare National Medical Reference Laboratory (NMRL) and Mutare Provincial Hospital Laboratory. DBS specimen collection at the clinics is done at 6 weeks’ post-natal visit and is integrated with the first Zimbabwean Extended Programme on Immunisation (EPI) Post-Natal Schedule. Turnaround times of results of the DBS to the clinics is harmonized with the second national EPI scheduled visit at 10 weeks of age for the baby which translates to a maximum period of 4 weeks.

The specimen rejection rates reported in literature vary widely across the world. In a 2011 study, Ciaranello et al reported that 16% out of 588 specimens collected were rejected due to improper labelling or missing information [16, 17]. This is far higher than the average specimen rejection rate (1-3%) for EID specimens reported amongst 26 low and medium income countries (including Zimbabwe, Angola, Botswana, Brazil, Burundi, Cameroon, Chad, China, Cote d'Ivoire, DRC, Ethiopia, Ghana, India, Kenya, Lesotho, Malawi, Mozambique, Namibia, Nigeria, Rwanda, South Africa, Swaziland, Tanzania, Uganda, Vietnam and Zambia) [14, 15]. A country specific rejection rate for EID specimens in Zimbabwe is not well described in literature.

There have been many factors associated with specimen rejection. According to reports reasons for specimen rejection include delays in sending the DBS samples for PCR testing, some specimen being improperly packed, amount of specimen being insufficient for running due to improper collection, specimens not being properly labelled with patient's name, patient's hospital number and the name of the collection clinic [18]. Some factors include specimens which appear diluted, have alcohol halo or serum ring around them and specimens which appear abraded, over-saturated, clotted, caked or layered, samples that appear discoloured or contaminated, samples not allowed to dry completely before packaging and mailing and specimens received without a patient/test request form. Specimen rejection may also be because of scientists not processing the specimens on time due to a few laboratory personnel trained on using the PCR machines [18, 19, 20]. Mutare Provincial Hospital is one of three EID diagnostic centres in Zimbabwe and there is dearth of information on specimen rejection rate and factors associated with rejected specimens at the EID laboratory.

MATERIALS AND METHODS

This was a cross-sectional descriptive study conducted among records of specimens submitted at Mutare Provincial Hospital Molecular Diagnostics Laboratory between January 2015 and December 2015. The records included date of specimen collection; date specimen was received at the laboratory; specimen suitability for analysis (accepted or rejected) and reasons for specimen rejection.

ETHICAL CLEARANCE

Ethical clearance was given by the Africa University Research Ethics Committee (AUREC)
based on ethical principles in the Declaration of Helsinki.

**STUDY DESIGN**

Cross sectional study

**STUDY PARTICIPANTS**

The study was carried out on data collected from early infant diagnosis patients for specimens referred to Mutare Provincial Hospital.

**METHOD OF DATA ANALYSIS**

Normally distributed data was summarized using means at 95% confidence interval and skewed data was summarized using median and interquartile ranges and frequencies. Data was analysed using Stata® statistical package (Texas, United States of America) version 10.1.

**RESULTS**

The specimen rejection rate was 10.7% (n=1472/13748) (Figure 1). The major reasons for rejection were improper sample collection 24.8% (365/1472) (Table 1), no specimen received 12.6% (185/1472), multiple specimens packed in one sample pack 17.2% (253/1472), specimens and forms not labelled correctly 8.5% (125/1472), specimens tested that requested for a new sample due to machine error hence rejected during testing 1.6% (244/1472), samples tested but failed 8.2% (120/1472) and samples tested with an indeterminate result 12.2% (180/1472).

![Sample rejection rate](image)

Figure 1 showing sample rejection rate

<table>
<thead>
<tr>
<th>FACTORS</th>
<th>NUMBER OF SPECIMENS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improper specimen collection</td>
<td>365</td>
<td>24.8</td>
</tr>
<tr>
<td>No specimen received</td>
<td>185</td>
<td>12.6</td>
</tr>
<tr>
<td>Multiple specimens packed in one sample pack</td>
<td>253</td>
<td>17.2</td>
</tr>
<tr>
<td>Specimens and forms not labelled correctly</td>
<td>125</td>
<td>8.5</td>
</tr>
<tr>
<td>Specimens tested that requested a new sample due to machine error</td>
<td>244</td>
<td>16.6</td>
</tr>
<tr>
<td>Specimens tested but failed</td>
<td>120</td>
<td>8.2</td>
</tr>
<tr>
<td>Specimens tested with an indeterminate result</td>
<td>180</td>
<td>12.2</td>
</tr>
<tr>
<td><strong>Total Number of Rejected Specimens</strong></td>
<td><strong>1472</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Grand Total of Received Specimens during period of study</strong></td>
<td><strong>13748</strong></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION AND RECOMMENDATIONS**

Reported rates of un-evaluable specimens vary widely, from 0.03% to 16% across the world [17]. Rate of specimen rejection at Mutare Provincial Hospital EID Laboratory for the period January-December 2015 was unacceptably high when compared to the range of 0.03%-16% reported by Ciaramello et al. and the average of 1-3% reported by CHAI for 26 countries including Zimbabwe [15]. Increased focus on site-based EID training and mentoring activities can assist in future decline in DBS specimen rejection rates observed [18].
The majority of the specimens in our study were rejected due to improper collection, multiple specimens being collected and packed in one sample pack factors attributable to personnel error. Some of the errors were analytical for example some specimens were tested but requested for a new sample due to machine error hence rejected during testing, some were tested but failed and some samples were tested with an indeterminate result. This suggests that local programs should intensify monitoring of pre-analytical and analytical staffs processes and performance towards improving specimen retention. Lack of standardized protocols for laboratory processes including specimen collection, specimen acquisition, management and storage could have contributed to errors in diagnostics. Implementing standardized protocols for reporting and managing non-conformance events could help improve service performance [18]. There is need for greater attention to specimen quality, clear guidelines on the responsibility and protocols for specimen collection and error reporting [18].

There is need for an integrated multidisciplinary approach which engages social support groups, health personnel, quality improvement interventions as well as electronic and mobile communication tools needed to improve uptake of PMTCT services and the overall health outcome of HIV positive mothers and their infants [18, 21]. Intensified training and monitoring of personnel, quality policies for specimen 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 counselling and active tracking of mothers and care givers to improve patient retention and achieve the goals of PMTCT programs [5,18, 21].

Not only the general community and care-givers should be well-educated about EID, but health personnel manning clinics should appreciate its potential benefits. These benefits range from improved access for HIV-exposed infants majority of who are HIV-negative to giving comfort to their care-givers. It is well established that documented infant HIV status permits assessment of PMTCT program effectiveness. Furthermore, providing evidence that large proportion of HIV-exposed infants is uninfected has potential to improve morale among PMTCT program staff [5, 20]. Accurate and early EID also informs infant feeding decisions in settings where breastfeeding is recommended for improved infant health. A diagnosis of HIV infection also permits discontinuation of postnatal antiretroviral prophylaxis, reducing the risk of drug-resistant virus associated with these medications [20]. Hence scientists have a significant role to play in the success of EID programs world-wide and indeed in our own context, by taking due care of training of colleagues involved in pre-analytical and analytical steps in the EID cascade. The results of the current study suggests real need for improvement at the specimen collection sites through rigorous training of clinicians and staff who collect, package and transport specimens and the laboratory staff who test to avoid human error.

REFERENCES


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