Delays in HIV-1 infant PCR testing may leave children without confirmed diagnoses

by

Kamela Lawrence Mahlakwane

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Department of Pathology

Faculty of Medicine and Health Sciences

Stellenbosch University

Primary supervisor: Prof. Gert van Zyl

Co-supervisors: Prof. Wolfgang Preiser

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Declaration

By submitting this thesis electronically, I, Kamela Lawrence Mahlakwane, declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Ke re "Montshepetša bošego wa bo rare, re mo leboga bosele! Ke le rata rati!"

Dedication

This paper is dedicated to all infants born to HIV infected mothers.

Abbreviations

AIDS	Acquired immunodeficiency syndrome
AJLM	African Journal of Laboratory Medicine
ART	Antiretroviral therapy
CAP/CTM	Roche® COBAS® AmpliPrep/COBAS® TaqMan®
cART	Combination antiretroviral therapy
Ct	Cycle threshold
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EID	Early infant diagnosis
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
HREC	Health Research Ethics Committee
IQR	Inter-quartile ratio
IT	Information technology
LIS	Laboratory information system
MRN	Medical record number
NHLS	National Heath Laboratory Service
PCR	Polymerase chain reaction
PMTCT	Prevention of mother-to-child transmission
RFI	Relative fluorescence intensity
RNA	Ribonucleic acid
SOP	Standard operating procedure
TAT	Turn-around time
WHO	World Health Organization

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Extended background

This thesis is presented in a publication-ready manuscript format, from an original research project that has been submitted to a scientific journal for publication. The revised, peer-reviewed version of the manuscript was prepared according to the African Journal of Laboratory Medicine (AJLM) author guidelines, and is now undergoing a second round of peer review, following a revision based on reviewers' feedback. We attach AJLM author guidelines as Appendix A.

In this manuscript, we report the findings resulting from a retrospective review of Human Immunodeficiency Virus (HIV) polymerase chain reaction (PCR) testing for early infant diagnosis (EID) in National Health Laboratory Service's (NHLS) Tygerberg virology laboratory.

Since the first cases of acquired immunodeficiency syndrome (AIDS) were reported in the United States 40 years ago[1], and subsequently linked to the infection with HIV[2], many people were infected, with current global prevalence at 37.9 million people, including 1.7 million children[3]. Prevalence of HIV infection among pregnant women in South Africa skyrocketed from 0.8% in 1990 to around 30% a decade and half later, stabilizing at that figure thereafter[4].

Many advances were since made in relation to prevention, diagnosis and management of HIV infection[5] in both adults and children. EID became part of national priority programmes in many countries including South African, to provide prevention of mother to child transmission (PMTCT) services[6, 7], with the roll-out of the PMTCT program beginning in 2002 in South Africa[4, 8]. This was initially used as a single-dose regimen of nevirapine in HIV infected pregnant women and exposed infants[9]. This was later proved to lead to drug resistance[10, 11], leading to improved and better drug regimen[12].

In the absence of antiretroviral treatment (ART) and other PMTCT measures, around 30% of HIV-exposed infants were expected to become HIV infected by age 4-8 weeks[4]. However, since improvement of drug regimen and PMTCT programmes, new paediatric HIV infections decreased by 84% between 2009 and 2015 in South Africa[7, 13]. The improvement of these drug regimen resulted in high rates of virological suppression in HIV-infected infants.[14-17]. While this is a great milestone in the management of HIV infection,

the rapid decay of HIV nucleic acid has led to diagnostic challenges due to loss of HIV nucleic acid detectability[14-17].

The NHLS has been tasked with providing laboratory testing for the public health sector, providing healthcare services for approximately 85% of the population who do not have medical insurance[18]. The NHLS performs all testing for the early infant diagnosis (EID) of HIV infection in children for the public sector, using the Roche® COBAS® AmpliPrep/COBAS® TaqMan® system (CAP/CTM) (Roche® Molecular Systems, Inc., Branchburg, NJ), in a network of centralized laboratories[18].

EID testing schedule has been a subject of constant evolution, both on World Health Organisation (WHO) and country levels[19-21]. Birth PCR is performed to detect intra-uterine infection, with subsequent tests done to detect any missed intra-uterine infection, perinatal or post-natal infections. These testing schedules may, however, differ slightly between WHO and the countries' national guidelines[19-21], with the latter putting more emphasis on birth PCR testing[20], and the former on the 6 week testing[21]. All the guidelines do recommend confirmatory testing on a separate blood sample for children who have been tested positive or negative[20, 21].

In this study, we assessed the proportion of rejected infant HIV PCR requests, established whether our laboratory met the required turnaround (TAT), and whether reactive infant HIV PCR results performed on our current HIV PCR testing platform triggered the prescribed confirmatory testing. This was achieved by describing and measuring the overall EID testing process from sample collection, reception, analysis, result and follow-up test requests over a two year period (July 2017 to June 2019). Both the good and the not-so-good findings were reported on, followed by the recommendations.

Chapter one introduces the topic and elaborates in detail the literature review related to EID and the importance of early initiation of ART in infants. We also describe the PCR testing platform that is used in our laboratory, further explaining the cut-offs used to determine positive and indeterminate results based on the initial irreproducible results in the earlier version of the assay[22, 23], and in the context of previous PMTCT programme[14]. These cut-offs were later revised[24-26].

Due to the centralized nature of EID services, many samples are referred from other facilities for HIV PCR testing. These samples do require transportation to the laboratory, which adds up to delayed TAT. The current national set standard for HIV PCR TAT in NHLS laboratories is 96 hours, which is equivalent to 4 days. This TAT definition only considers the time within laboratory processes, without taking into account the period between sample collection and reception, and the period between the time the results are authorised by the virologist to the time they are received by the patient.

This may add to the delay for follow-up testing in patients whose samples tested negative or indeterminate, and thus to proper patient management. Point-of-care testing platforms have been piloted for HIV-1infant diagnosis and can be beneficial in improving TAT, but require task shifting, generally have limited throughput and relative high cost and often have relative high levels of complexity, requiring trained personnel[27].

Chapter two details the material and methods of this study, including study design, setting, sample, data collection and ethical consideration. The study findings are reported in chapter three and discussed in detail in chapter four, with both limitations and recommendations, followed by conclusion in chapter five. Due to the high number of initial reactive tests, without evidence of follow-up, we suggested that a shorter TAT would be required to allow confirmatory testing, before children are discharged from post-natal wards.

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Publication-ready manuscript

The following manuscript has been prepared for, and re-submitted to African Journal of Laboratory Medicine (AJLM) as a second version of the revised peer-reviewed manuscript, based on the reviewers' feedback. The journal's aims and scope, as well as author guidelines are given in Appendix A.

Delays in HIV-1 infant PCR testing may leave children without confirmed diagnoses

Full author details

Kamela L. Mahlakwane, Medical Virology registrar, Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa. Email: kamela.mahlakwane@nhls.ac.za. ORCID ID: https://orcid.org/0000-0002-7568-9098

Wolfgang Preiser, Professor and Head, Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa. Email: preiser@sun.ac.za. ORCID ID: https://orcid.org/0000-0002-0254-7910

Nokwazi Nkosi, Consultant Virologist, Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa. Email: nokwazi@sun.ac.za. ORCID ID: https://orcid.org/0000-0002-4554-180X

Nasheen Naidoo, Clinical Pathology registrar, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa. Email: nnaidoo@outlook.co.za. ORCID ID: https://orcid.org/0000-0001-8613-4808

Gert van Zyl, Professor and Consultant Virologist, Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and National Health Laboratory Service, South Africa. Email: guvz@sun.ac.za. ORCID ID: https://orcid.org/0000-0003-3021-5101

Corresponding author
Kamela L. Mahlakwane

Address: Division of Medical Virology, 08th Floor Clinical Building, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town, South Africa, 8000.

Tel: +27 21 938 9691

Email: kamela.mahlakwane@nhls.ac.za

Abstract

Background

The early diagnosis and confirmation of HIV infection in newborns is crucial for expedited antiretroviral therapy initiation. Confirmatory testing must be done for all children with a reactive HIV PCR result. There is no comprehensive data on confirmatory testing and rejection of HIV PCR test requests at National Health Laboratory Service laboratories.

Aim and objectives

To assess relevant measures for routine infant HIV PCR testing: rate of rejected test requests, turnaround time, and rate of confirmatory testing.

Method

A retrospective review was performed on the laboratory-based data of all HIV PCR tests that were performed on children ≤24 months old (n=43,346), and data of rejected HIV PCR requests (n=1,479) over a two-year period (2017-2019). These data were extracted from the laboratory information system. Data were analyzed from sample collection to release of results, assessing the TAT and follow-up patterns.

Results

The proportion of HIV PCR requests that were rejected was 3.3%, of which 83.9% were rejected for various pre-analytical reasons. The majority of test results (89.2%) met the required 96-hour TAT. Of the reactive initial test results, 53.5% had a follow-up sample sent, of which 93.1% were positive on follow-up. Of the initial indeterminate results, 74.7% were negative on follow-up.

Conclusion

A significant proportion of HIV PCR requests were rejected for various pre-analytical reasons. The high number of initial reactive tests, without evidence of follow-up, may suggest that a shorter TAT would be required to allow confirmatory testing, before children are discharged.

Chapter 1: Introduction and literature review

The early diagnosis of Human Immunodeficiency Virus (HIV) infection in infants is very important[1, 2]. Infants who are initiated on antiretroviral therapy (ART) within 7 days of life are four times more likely to achieve early viral suppression than those whose ART commences later[3, 4]. The South African national guidelines on the prevention of mother-to-child transmission (PMTCT) of HIV recommend that HIV PCR test results be checked within 3-6 days postnatally, and ART be immediately initiated in all children with detected HIV nucleic acid, while awaiting the follow-up HIV polymerase chain reaction (PCR) results[5].

Early initiation of ART is associated with improved outcomes and reduced morbidity and mortality[6-8]. Not only does early initiation of ART result in viral suppression, but also has better clinical and immunological outcomes[9]. Earlier diagnosis of HIV-infected infants facilitates earlier access to therapy and improved clinical outcomes[10]. It is dependent on the earliest possible diagnosis of HIV infection, which is done by testing for viral nucleic acid (DNA and/or RNA) in blood samples, usually by PCR[11].

Current guidelines, including those issued by the Western Cape Provincial and South African National Department of Health recommend that all HIV-exposed infants have blood samples tested for HIV nucleic acid by PCR within 7 days of birth (i.e. birth PCR); at approximately 10 weeks of age for those that tested negative at birth; at 18 weeks for high risk infants who received extended prophylactic ART for 12 weeks; at month 6 of life; and finally, after cessation of breastfeeding[5, 12, 13]. The post-breastfeeding cessation HIV test may either be by PCR if the child is younger than 18 months of age, or by serology if the child is older[5, 11, 12].

World Health Organization (WHO) recommends that HIV antibody testing should be undertaken at least 3 months after cessation of breastfeeding (to allow for development of HIV antibodies), since infection acquired in the last days of breastfeeding may be missed[13]. If breastfeeding extends beyond 18 months, the final diagnosis of HIV status can only be assessed at least 3 months following cessation of breastfeeding. If the infant is older than 18 months, negative antibody testing confirms that the infant is uninfected[13].

Although WHO guidelines recommend HIV PCR testing for infants who are 18 months of age and younger, it has been National Health Laboratory Service (NHLS) laboratories'

internal practice that the age cut-off for HIV PCR testing be 24 months rather than 18 months. This is based on studies that showed clearance of maternal HIV antibodies taking more than 18 months in some perinatally exposed infants, with seroreversion rates of 89.3%, 94.2% and 100% at 12, 18 and 24 months of age, respectively[14, 15]. Nevertheless, this policy has not been formalized in the laboratory diagnostic booklet, the Western Cape provincial, or the National Department of Health HIV testing guidelines.

Given the consequence of a diagnosis of HIV infection (namely, lifelong ART), it is consensus that a follow-up sample be collected as soon as possible from all children with a reactive infant PCR result, to confirm the diagnosis of HIV infection, and to detect false positive initial results[11, 16]. However, a negative test result following initial reactive result does not necessarily constitute false positive, particularly in infants who are on prophylaxis or ART.

False positive results need to be identified expediently. Clinical action taken on the basis of a false positive result may have dire repercussions such as biomedical (exposure to drug toxicity and side effects), psychosocial (possible stigma, life-outlook, future relationships), financial (costs of ART) and medico-legal implications[16]. Once the child is on ART, it may become impossible to distinguish someone virally suppressed from someone who has never been infected in the first place[4]. This is a very important challenge that confronts many diagnostic laboratories, as rapid decline of HIV-1 nucleic acid may complicate definitive diagnosis when confirmatory testing is delayed[4].

In South Africa, the NHLS provides laboratory testing for the public health sector, providing healthcare for approximately 85% of the population who do not have medical insurance[17]. The NHLS aims to issue results for at least 80% of infant PCR tests within a 96 hour turn-around time (TAT)[18]. This was calculated based on the NHLS definition[19] as the time interval from sample reception to the time the final results were released by a virologist. This TAT, therefore, only includes the time period within laboratory processes, and thus, does not take into account the periods between sample collection and reception, and from reviewing of results to the time the patient actually receives them.

The NHLS laboratories perform all testing for the early infant diagnosis (EID) of HIV infection in children for the public sector, using the Roche® COBAS®

AmpliPrep/COBAS® TaqMan® system (CAP/CTM) (Roche® Molecular Systems, Inc., Branchburg, NJ), in a network of centralized laboratories[17].

The possible results generated from this assay can be one of the following three: positive, negative, or indeterminate. Indeterminate qualitative HIV PCR results when using CAP/CTM were first described in our laboratory in 2012[20, 21]. During the period covered by this study, indeterminate results were defined as a cycle threshold (Ct) of 33 or above, and/ or a relative fluorescence intensity (RFI) below 5[22].

These diagnostic criteria have since been revised[23-26], mostly based on collated data, of which cut-offs were most predictive of reproducibly positive results, where Ct proved to be the only predictive variable, with Ct <33.0 found to be the most accurate threshold value for differentiating clearly positive from irreproducible cases[23].

In our laboratory, HIV PCR testing is done only once on each sample, unless the results are invalid, in which case a single retest is done on the remnant sample. Samples that produce invalid results for the second time are rejected with this reason attached: "failed after repeated attempts". All other results (i.e. negative, positive, or indeterminate) are released as such, with no repeat testing of the remnant sample.

There is currently no available confirmatory assay for HIV PCR testing in our laboratories. If indeterminate samples were to be repeated, this would unlikely be any beneficial in resolving the uncertainty of the results, as the same sample would be tested on the same platform. A repeat test that produces negative results would still not be regarded as negative, due to the possibility of low nucleic acid concentration, low input volumes or dilution factor[27]. Over and above these many dependencies between repeat test results, repeat testing's main disadvantage may be an additional delay in reporting an uncertain result that in any case requires an independent sample for confirmation.

WHO reports that most women and their newborn infants are likely to be discharged within 1 to 2 days following uncomplicated vaginal delivery, and within 2 to 4 days following uncomplicated caesarean section[28]. Thus, infant birth HIV PCR results should, ideally, be available before the child gets discharged home, to allow immediate optimal management (prophylaxis, ART, and/or confirmatory testing). HIV PCR TAT strategy should, thus, seek to address this challenge.

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In this study, a laboratory-based retrospective review was conducted to assess the proportion of rejected infant HIV PCR requests, to establish whether our laboratory meets the required TAT, and whether reactive infant HIV PCR results performed on the CAP/CTM triggered the prescribed confirmatory testing. This was achieved by describing and measuring the overall EID testing process from sample collection, reception, analysis, result and follow-up test requests. The time period analysed for this study was from July 2017 to June 2019.

Chapter 2: Methods

Study design

This is a retrospective review using Tygerberg virology laboratory data generated between July 2017 and June 2019 and downloaded from the laboratory information system (LIS), TrakCare Lab (Intersystems Corporation, Cambridge, Massachusetts, United States).

Study setting

The laboratory renders diagnostic virological pathology services to Tygerberg hospital and other health facilities from its drainage areas. All samples received in the laboratory go through the routine process of sample reception, registration, testing, analysis, and reviewing of results. This intra-laboratory process was the focus of the laboratory's TAT. The periods between sample collection and reception in the laboratory and from results authorization until the patient receives the results did not form part of the official TAT calculation as per the NHLS definition.

Study sample

All results on ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood and dried blood spot (DBS) samples from children 24 months of age and younger that were tested by HIV-1 qualitative PCR at Tygerberg virology laboratory between 01st July 2017 and 30th June 2019 were included. Excluded were samples from patients older than 24 months (n = 534) or with unknown age (n = 287), quality control samples (n = 29) and samples with coded names or surnames (n = 15). The resulting tested HIV PCR dataset contained 43,346 tests.

Data collection

i. *Rejection of test requests*

A raw dataset of rejected HIV PCR requests for 24 months old children and younger were extracted from LIS (n = 1,479).

We assessed these samples for the patterns of HIV PCR request rejections by the laboratory. Infant PCR test requests may be rejected for various reasons, as included in the LIS and NHLS standard operating procedure (SOP) for sample rejection. These reasons may be pre-analytical, such as "unsuitable age" for children older than 24 months of age, or analytical, such as "laboratory error".

Samples that come from other peripheral laboratories are referred both digitally and physically. Digital referrals that were not accompanied by the sample were rejected as "lost in transit" or "specimen not received" after consultation with the referring laboratory or the requesting clinician. All EDTA samples are discarded after 7 days from date of collection. Samples that have not been tested within this period are rejected as "sample too old".

In other cases, the term "insufficient specimen" could have been used for the samples that could have already been tested - with initial "invalid results" – thus resulting in insufficient remaining sample volume to repeat the test. The classification of these rejections as pre-analytical is, therefore, arbitrary. All "invalid results" are supposed to be classified as analytical, as they all would have already been tested.

ii. *Turnaround time (TAT)*

TAT was calculated based on the NHLS definition[19] as the time interval from sample reception to the time the final results were released by a virologist. These time points are extracted from LIS as recorded during each step of the testing process. The results were then stratified into those that had a TAT below and above 96 hours, respectively.

iii. *Follow-up Testing*

All results were grouped together using the unique laboratory code, a medical record number (MRN), assigned to each individual patient that remains the same for all subsequent test requests. Time to follow-up was calculated as the number of days between the first and second sample collection dates as recorded on the LIS. In line with the guidelines, all positive and indeterminate results were expected to have a follow-up sample sent for

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confirmatory testing as soon as possible, while all the negative samples were expected to be followed up as per schedule. For those follow-up results that were negative, we went further and checked whether there was a third follow-up sample that was tested

iv.   *Data analysis*

Data were analysed using Microsoft Excel 2010 version 14 (Microsoft Corporation, Redmond, Washington, United States). Rejected test requests were stratified by reasons for rejection, and further summarised into pre-analytical and analytical reasons for rejection.

We initially categorised the results into age, gender and outcome (negative, positive, or indeterminate). We then analysed the TAT of each result, and categorized them into those that were resulted within 96 hour period, and those beyond this time period. We also assessed whether the initial results received follow-up testing as per guidelines, and the lapsed days between initial results and the first follow-up sample collection.

Ethical considerations

This study was approved by the Health Research Ethics Committee (HREC) of the University of Stellenbosch (ref. no. S19/03/053). A waiver of informed consent was obtained, as this study did not enrol human participants. Access to extracted laboratory data was limited to the researchers only.

Chapter 3: Results

Rejected test sets

Of the 44,825 HIV PCR test requests of children 24 months old and younger, 43,346 (96.7%) were tested, with 1,479 (3.3%) of the samples being rejected for various reasons. Of these, 1,241 (83.9%) and 238 (16.1%) were rejected for pre-analytical and analytical reasons, respectively (Figure 1). Duplicate requests (21,3%), insufficient specimen (21,1%), and specimen not received (16,1%) formed the bulk of pre-analytical reasons for sample rejections (Table 1). Rejection reasons that were less than 1% were grouped together into "other various reasons" (Table 1).

Demographics

Of the 43,346 samples that were tested for infant HIV PCR, 27,978 (64,6%) were the patients' first or initial HIV PCR samples, with 18,393 (42.4%) having been collected at

birth. Amongst those tested for the first time after seven days of life (i.e., beyond birth PCR), 5,715 (59.6%) were tested within a 10 week timeframe (1<weeks>12) with a median age of 10 weeks [inter-quartile ratio (IQR) = 8-11]. There was no gender bias, with samples from both males and females at 49.9% and 49.8%, respectively.

Turnaround time

A total of 38,653 (89.2%) test results were signed out within 96 hours from sample reception (median time = 44 hours (IQR = 31-64), while the remaining 4,693 (10.2%) did not meet the desired 96 hour TAT, with median time of 116 hours (IQR = 106-137). This is the TAT that is defined in the NHLS national set standards.

However, the total TAT from sample collection to review of results revealed that only 33,245 (76.7%) samples were signed out within 96 hour TAT with a median time of 57 hours (IQR = 47-73), while 10,101 (23.3%) were released beyond this time period with median time of 119 hours (IQR = 105-140). Sample collection to sample registration had a median TAT of 17 hours (IQR = 8-24).

Instrument downtime events due to power failure, technical problems, and information technology (IT) related issues during which there were no results transmitted from the infant PCR testing platform to the LIS were noted. However, there was no data at hand to assess how these may have impacted on the variability of the TAT.

Follow-up HIV PCR test results

Of the 520 (1.9%) reactive initial results, approximately half were followed up by testing of a subsequent sample (Figure 2). Likewise, only 9,409 (50.3%) of the initial negative results had subsequent samples tested, with only 1,264 (13.4%) of these being done at 10 weeks of age (6 – 12 weeks of life) as per the national HIV testing guidelines. Nine (0.71%) of these 10-week sample results had positive or indeterminate results. It should be noted that some of the follow-up results may have not been linked to the initial samples, and thus been missed in this analysis.

Of the 520 initial reactive tests, 251 (48.3%) were done at birth, while 269 (51.7%) were tested later in life (>7 days of life). The confirmatory testing proportion between these age groups were 62.9% (158) and 44.6% (120) for the former and the latter, respectively.

The reactive results were further analysed to assess the agreement between initial and confirmatory results (Table 2). Of the patients with indeterminate results for their first sample that had a confirmatory sample tested, 56 (74.7%) had negative results (Table 2). Of these, 30 (53.6%) had a third HIV PCR follow-up test done, while 24 (42.9%) of the results having no third follow-up test, whether in a form of HIV PCR or HIV viral load (Table 3). Amongst the 30 third HIV PCR tests, 29 (96.7%) were negative (Table 3). There were two results that had no HIV PCR follow-up test, but had HIV viral load done. One of these had detectable HIV viral load (862,328 copies per millilitre). The presence of detectable HIV nucleic acid in this quantitative molecular test is confirmatory of HIV infection.

However, 189 (93.1%) patients with positive results for their first sample were also positive following confirmatory testing, while five (2.5%) were indeterminate (Table 2). This is a 95.6% reactivity on confirmatory testing, with nine (4.4%) discordant results (negative on follow-up testing). Further scrutiny of these nine results revealed that all of them had a third follow-up HIV PCR tests, with four testing positive, while five remained negative.

Urgency of follow-up samples

Of the 278 (53.5%) follow-up tests that were done, 147 (59.3%) were performed more than 7 days following the initial test (Average = 82 days; Range: 8 – 606 days). Amongst the 131 (47.1%) of the follow-up samples that were performed within the first 7 days of life, only 52 (18.7%) samples were within 3 days after the initial test.

Chapter 4: Discussion

Of all the infant PCR requests, 3.3% were rejected for various reasons. The top three reasons for sample rejection were due to requests that were duplicated, sample volumes that were insufficient for testing, and specimen that were lost in transit. These rejections happen when, for instance, more than one clinician requests the same test for the same patient, the sample is received in the laboratory with a volume lower than that required for the test, or test requests that are not accompanied by the patient's sample.

This proportion of rejected samples is concerningly high, and does impact negatively on early diagnosis and management of HIV infected infants. Comparative data from other

settings is limited, thus making it difficult to determine the rejection threshold across laboratories.

An overall appraisal of the TAT shows that our laboratory meets the standards set by NHLS National Strategic Plan. However, narrowing in on the positive and indeterminate results, which are the focus of our study, we note that the average time from sample reception to authorisation of results is prolonged, with 14.9% of these results having been released after the required 96 hours TAT.

It should also be noted that the 96 hour TAT - which is equivalent to 4 days – only includes the processes inside the laboratory, and does not take into account the time from sample collection to reception, and from dispatch of results until the patient receives them. The TAT from sample collection to results review has proven to be even longer, with only 76.7% of results being released within 96 hour TAT. Again, this does not take into account the period from results authorization to the time the patient receives the results. This TAT might require some revisions to align with clinical relevance of expediting EID and early initiation of ART. Point-of-care HIV PCR testing has been shown to achieve same-day diagnosis for infants, and help with rapid ART initiation[29].

A case in point is the 18.7% of the reactive confirmatory tests that were done within 3 days after the initial test. On average, the majority of newborns would have been discharged from hospital by day 3 of life. If the results are received within this time period, newborns with positive birth PCR are likely to be discharged on ART, which would reduce the number of patients lost to follow-up[29]. A quicker TAT would help with early initiation of ART for the newborns who would still be in hospital at the time the results are received.

Mothers of the newborns are required to visit the clinic within 6 days after birth. The 6-day maternal postnatal clinic visit was reported to be at 58.0% in the Western Cape during 2017-2019 time period[30]. The 59.3% delay in follow-up samples beyond 7 days may be as a result of this low uptake. But again, this may also be due to the fact that our 96 hour TAT only takes into account the processes within the laboratory, which might contribute to the delays in confirmatory testing.

While 1.9% of initial test results were reactive, only 53.5% of these had the prescribed confirmatory test. It is unknown whether the other 46.5% of reactive results were ever acted

upon. If not, this may mean that many HIV infected patients would have not been initiated on ART, or alternatively, a substantial proportion would have been started on ART without confirmatory test results, effectively exposing some uninfected children to lifelong ART.

For those that had a confirmatory sample, 95.6% were reactive on follow-up. Amongst the 9 (4.4%) discordant results, only four became positive on third follow-up tests, with five samples remaining negative. This could either indicate that these initial reactive results were false reactive in the first place, or may be as a result of rapid decline in HIV nucleic acid concentration secondary to exposure to highly active ART (HAART), in infants who are receiving antiretroviral drugs (as PMTCT or cART). The dilemma in this situation is that we might not be able to make a definitive diagnosis of HIV infection in these children.

The majority of children with indeterminate results tested negative on follow-up, which again proved negative on third follow-up test. Again, this could either indicate that a large proportion of these indeterminate results were false reactive in the first place, or may be as a result of rapid HIV nucleic acid decay in children who are receiving ART for prophylaxis, which in high risk cases may be two or three drugs[4, 31]. It should, however, be noted that the indeterminate cut-offs used in this study were based on reproducibility on a particular assay, with a particular chemistry and software, and within a different PMTCT context. These were later improved. Recently, HIV diagnostic criteria have been revised with the aim to address this diagnostic dilemma[23-26].

Only 13.4% of patients with negative birth PCR received a 10-week follow-up test as per schedule, although about half (50.3%) were followed up later in life. This is in keeping with other findings from similar local studies on 10-week HIV PCR follow-up tests[32]. At 10-weeks, 0.7% of infants tested HIV PCR positive or indeterminate, which is lower than the national HIV prevalence of 0.9%, and the National Strategic Plan target of 1.3% at 10 weeks[30]. It is, however, higher than the reported Western Cape rate of 0.5% [30]. It should be noted that while these patients could have been missed at birth following intra-uterine infection, they also could have been infected either perinatally or postnatally.

The 53.5% follow-up test rate following an initial reactive infant PCR result is similar to the Tshwane study in South Africa, which reported a 53.0% confirmatory test rate from the laboratory data[33]. This proportion may be different from what is seen in clinical practice[33], as some of the patients may have had either HIV viral load or serological tests as a follow-

up, particularly for patients older than 18 months of age. These patients would have been missed in our study, as it focused specifically on HIV PCR results.

Limitations

Our study has several limitations. These include the interpretation of the TAT data, which needed to take into account the current workflow practice. HIV PCR testing is processed only during the weekdays (Monday to Friday). The fact that no tests are done on the weekends may result in variability of TAT. This study is thus not indicative of the laboratory that works on a shift system (i.e. 24hr service). Occasionally HIV PCR results fail to transmit to the LIS, further delaying the TAT.

While our TAT was at par with the national set requirements despite the fact that we only do routine HIV PCR tests on weekdays. The total TAT from sample collection to authorization of results – which may take few days – impacts on proper management of HIV infected infants. The magnitude of this challenge remains unknown, particularly in remote settings with poor access to laboratory diagnostic services.

Another limitation is that indeterminate results were classified using out-dated criteria that have since been revised. Some of these results may now be re-classified as positive.

Follow-up tests were reconciled using MRN number for each patient. However, we noted that some patients may erroneously have more than one MRN number. This could have occurred, possibly, for babies whose samples were collected and labelled with the mother's name (i.e. Baby of XYZ) and then later given their own names, resulting in non-reconciled patient profiles. This might have impacted on the under-reporting of follow-up tests, as these would have been missed. We, however, compared data provided on actual requests forms of the babies whose initial tests had "Baby of" as their first names, with those captured by LIS and found them to match well.

Also, some patients could have moved to other health facilities outside our testing site. It must be noted that this study was limited to Tygerberg virology laboratory testing site, and thus may not be representative of the whole Western Cape region (i.e. Groote Schuur hospital laboratory and Green Point Complex). The magnitude of this data concern is unknown, due to our inability to assess the number of samples that were not linked.

Recommendations

There was a high proportion of infant HIV PCR requests that were rejected for various reasons, majority of which were of pre-analytical nature. Just over half of infants who required confirmatory PCR testing received such test. It remains unclear whether the patients who were not followed up are HIV infected or not. A small proportion of babies received the 10-week follow-up test. These patients may only be seen in the hospital later in life when they are sick. Clinician education on HIV testing guidelines may help in reducing this number. Systems strengthening to reduce time between first and confirmatory HIV PCR tests could be beneficial.

Our TAT is at par with the national set requirements, despite the fact that we only do routine infant PCR tests on weekdays. However, this 96 hour TAT criterion might require some revisions to align with clinical relevance of expediting EID and early initiation of ART. Point-of-care HIV PCR testing has been shown to achieve same-day diagnosis for infants, and help with rapid ART initiation. This would be a useful alternative to resolve these delays.

Most positive results were reproducible on repeat. However, the majority of indeterminate results were negative on follow-up, and also following the third test. It was not known if patients were receiving PMTCT regimens or combination ART during the periods of observation, as that may have resulted in undetectable HIV PCR results. We hope the new early infant diagnostic criteria will help resolve the challenges presented by indeterminate HIV PCR results. A separate, independent HIV PCR testing platform may also assist in improving this.

Chapter 5: Conclusion

A significant proportion of infant HIV PCR requests were rejected for various reasons. Our laboratory's TAT is at par with the national set requirements. However, this 4-day intra-laboratory TAT may need to be revised as it may negatively impact on the number of children who receive confirmatory testing after a positive result. A larger study is recommended for clearer appreciation of the HIV PCR challenges.

Chapter 6

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Competing interests:

The authors declare no conflict of interest.

Authors' contributions

KLM: Conceptualization of the research project, investigation, analytical calculations, and writing of original draft.

WP: Supervised the project, and also helped with editing and reviewing of the final version of the manuscript.

NPN: Provided critical feedback and helped shape the research, data analysis, and editing of the manuscript.

NN: Helped with analytical calculations, drafting and editing of the manuscript.

GUVZ: Supervised the project, and also helped with editing and reviewing of the final version of the manuscript.

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The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Data availability statement:

The data that support the findings of this study are available on request from the corresponding author, KLM. The data are not publicly available due to their containing information that could compromise the privacy of patients whose samples were used in this research project.

Disclaimer:

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Stellenbosch University or NHLS.
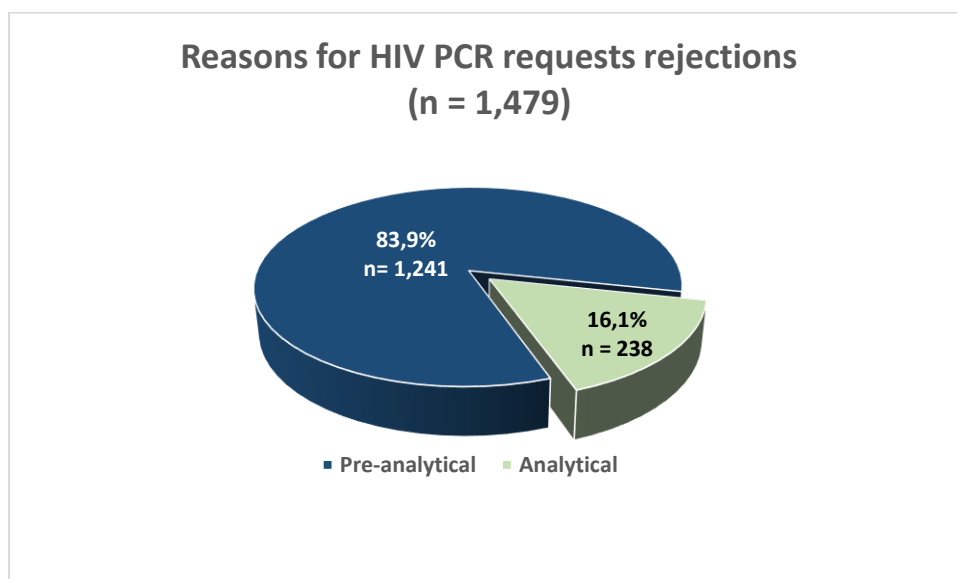
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Tables and figures

Reasons for HIV PCR requests rejections (n = 1,479)

83,9%
n= 1,241

16,1%
n = 238

■ Pre-analytical ■ Analytical

Figure 1: Reasons for HIV PCR test rejection

Table 1: Reasons for rejections of HIV PCR test requests

Reasons for rejection	No. of rejected requests	Percentages	Category
Duplicate request	315	21.3%	Pre-analytical
Insufficient specimen	312	21.1%	Pre-analytical
Specimen not received	238	16.1%	Pre-analytical
Require blood specimen	106	7.2%	Pre-analytical
Sample too old	50	3.4%	Pre-analytical
Specimen not labelled	50	3.4%	Pre-analytical
Information mismatch	46	3.1%	Pre-analytical
Container empty	43	2.9%	Pre-analytical
Invalid result	110	7.4%	Analytical
Failed after repeated attempts	109	7.4%	Analytical
Other various reasons*	100	6.7%	Mixture
Total rejected samples	1,479	100%	

*All rejected samples that formed 1% or less of the rejection reasons we grouped together into "Other various reasons". These rejection reasons are inclusive of both pre-analytical and analytical categories.
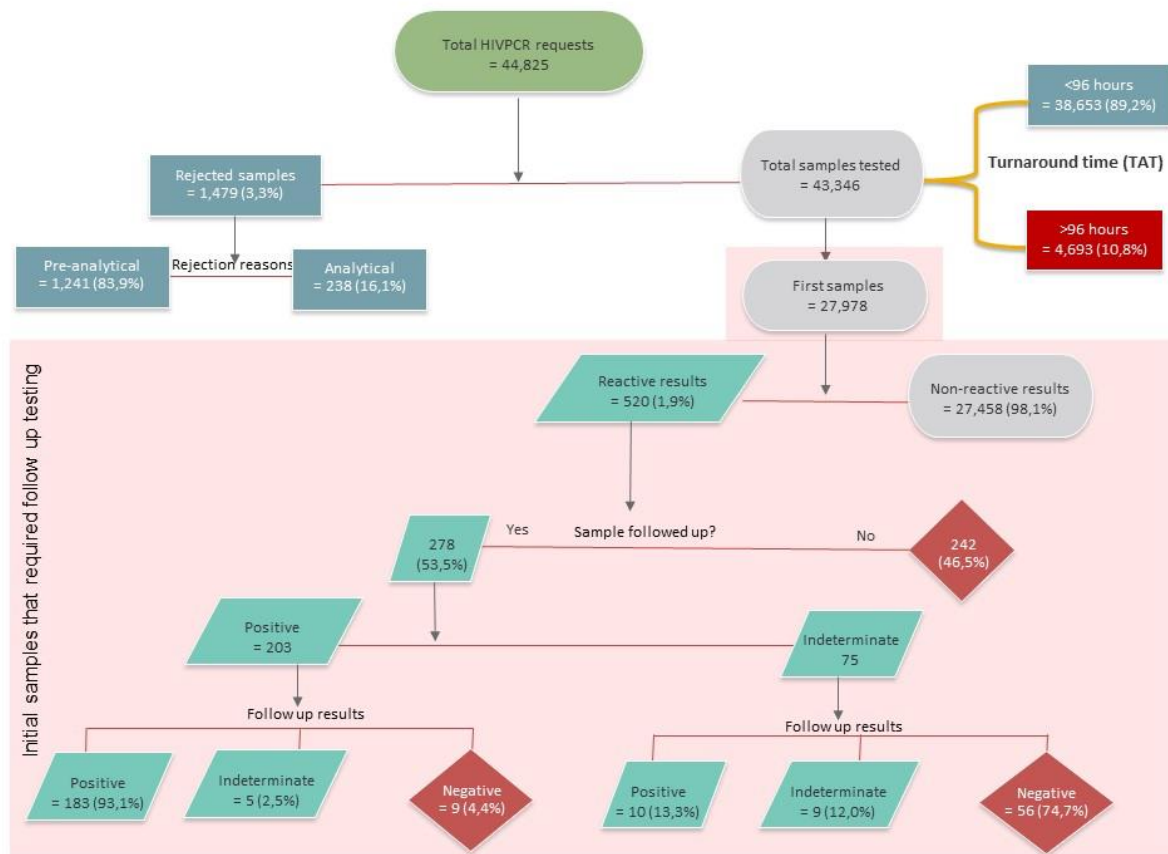
Figure 2: Total HIV PCR requests, rejected samples, turnaround time, and follow-up tests following initial (first) test. The follow-up tests are shaded.

Table 2: Comparison of the initial reactive infant PCR results and their confirmatory test results

Initial test results that were followed up	Confirmatory test results			Total
	Positive	**Indeterminate**	**Negative**	
Positive	189 (93.1%)	5 (2.5%)	9 (4.4%)	203
Indeterminate	10 (13.3%)	9 (12.0%)	56 (74.7%)	75
Total				**278**

Table 3: Third follow-up tests for samples that initially tested indeterminate, followed by negative or another indeterminate confirmatory results on second samples

1st & 2nd HIVPCR	Total	3rd HIVPCR done	Reactive 3rd HIVPCR	Non-reactive 3rd HIVPCR	No 3rd HIVPCR, no HIVVL done	No 3rd HIVPCR, but HIVVL done	No 3rd HIVPCR, but detected HIVVL	Both HIVPCR and HIVVL done	Non-reactive HIVPCR, but detectable HIVVL
IND + Negative	**56**	30	1	29	24	2	1	10	1
IND + IND	**9**	6	3	3	2	1	1	5	0

Appendix A – AJLM author guidelines

Appendix B – Ethics approval

Appendix C - Turnitin report

Appendix D - Plagiarism declaration

Appendix E - Original research protocol