



Evaluation of the Aptima HIV-1 Quant Dx assay for HIV diagnosis at birth in South Africa

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ABSTRACT

The increased coverage of antiretroviral therapy has resulted in a decrease in the positive predictive value (PPV) and diagnostic sensitivity of early infant diagnosis assays. To evaluate the diagnostic performance of the Aptima HIV-1 Quant DX assay (Aptima) in detecting HIV infection at birth. The study was a cross-sectional laboratory based evaluation using whole blood DBS specimens. Samples were collected from HIV-exposed neonates at birth at two paediatric facilities in Gauteng between 1st March 2018 – 31st January 2020. Performance of the Aptima compared to the Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0 was calculated using a two-by-two table and reported as proportions with 95% confidence intervals. A total of 363 infants met the inclusion criteria of which 4 (1.1%) had an Aptima result discordant with CAP/CTM HIV status: two (50%) negative and two (50%) positive. The Aptima assay had a sensitivity of 93.75% (95% CI: 79.19%–99.23%), specificity of 99.4% (95% CI: 97.83%–99.93%), PPV of 93.75% (95% CI: 78.98%–98.36%), negative predictive value of 99.4% (95% CI: 97.73%–99.84%), and overall accuracy of 98.9% (95% CI: 97.2%–99.7%). The Aptima yielded an error code on 37 (10.19%) results, of which 35 (94.59%) were resolved on repeat testing. Of the 32 HIV-detected specimens, 20 had a plasma VL result available (18 on Abbott and 2 on Cobas). The absolute median difference was 0.66 log₁₀ (IQR: 0.36–1.71). The Aptima demonstrated good EID performance and can be considered as a qualitative EID assay.

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1. Background

High maternal HIV prevalence in South Africa (SA) means that the number of infants and children infected with HIV remains high despite a low overall mother-to-child transmission rate of less than 5%. (Sherman, 2016) Because HIV disease progression occurs rapidly in infants, accurate early infant diagnosis (EID) of HIV is important in order to prevent HIV related morbidity and mortality. (Bulage et al., 2017, Kalk et al., 2018, Lilian et al., 2013) Infant diagnosis of HIV refers to virological testing performed on infants born to women living with HIV (WLHIV), in order to establish their HIV status. In SA, real-time polymerase chain reaction (RT-PCR) tests approved for *in-vitro* diagnostic use are predominantly used for early infant diagnosis (EID), this refers to testing performed prior to the infant reaching two months of age. All testing within the public sector performed by the

National Health Laboratory Service (NHLS). Since 2010 the NHLS has used EID assays from a single manufacturer, with the Cobas® AmpliPrep/Cobas® TaqMan (CAP/CTM) HIV-1 Qualitative Test version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) used since 2014. The NHLS applies a standard operating procedure (SOP) across all 11 EID designated laboratories. (Sherman et al., 2017) Routine testing is performed on whole blood collected either in an ethylenediaminetetraacetic acid (EDTA) tube or on a dried blood spot (DBS) card, the latter being the predominant sample type on account of ease of collection, storage, and transportation. (Rottinghaus et al., 2014)

On account of the prevention of mother-to-child transmission (PMTCT) programme, HIV-exposed infants are increasingly exposed to maternal and infant antiretroviral (ARV) drug regimens (Violari et al., 2008) which can cause a reduction in circulating HIV RNA and proviral HIV DNA levels, and a decrease in the HIV reservoir size among infected infants. (Mazanderani and Sherman, 2019) This has been associated with false-negative and indeterminate HIV PCR

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results. (Mazanderani et al., 2017) Indeterminate HIV PCR results are HIV-detected results with a low signal which could suggest a false-positive result. In lieu of using multiple consecutive assays to minimise the rate of false-positive results, the World Health Organization (WHO) recommends the use of an indeterminate threshold, which allows for differentiation of potentially false-positive results from those that are clearly positive. (World Health Organization 2018) Since 2013, this has been defined within the NHLS as a cycle threshold (Ct) >33 and/or a relative fluorescence intensity (RFI) <5 on the CAP/CTM assays. Indeterminate results account for approximately 17% of HIV-detected results in South Africa (Mazanderani et al., 2017, Mazanderani et al., 2016) with almost half of infants with an indeterminate birth PCR result found to be HIV infected on follow-up. (Technau et al., 2017) This highlights the need for all infants with an indeterminate result to be followed-up and testing repeated as a matter of urgency. The high volume of indeterminate HIV PCR results in South Africa also highlights the need to evaluate the performance of alternative EID assays within the context of antiretroviral therapy (ART) exposure and to determine whether the HIV status of infants who test indeterminate can be resolved by testing the same specimen on alternate assays prior to result-verification.

In addition to challenges with diagnostic sensitivity as outlined above, the positive predictive value (PPV) of EID assays is invariably declining on account of a decrease in the prevalence of paediatric HIV. (Burgard et al., 2012, World Health Organization 2015, World Health Organization 2015) Hence, a greater proportion of infants will receive false-positive results thereby putting infants at risk of commencing unnecessary ART regimens that could have negative medical and social consequences. (Dunning et al., 2017) A reduced PPV alongside viraemic suppression caused by ARV exposure represent significant challenges for accurate EID. (Mazanderani et al., 2017, Mazanderani et al., 2016) To effectively address these challenges South Africa may need to revise the current EID testing algorithm, which may include the introduction of multiple assays from different manufacturers.

In light of this, we evaluated the diagnostic performance of the Hologic Aptima HIV-1 Quant Dx assay (Hologic Gen-Probe, Inc., San Diego, CA), referred to herein as the Aptima, as an additional assay for testing on whole blood DBS for the neonatal diagnosis of HIV.

2. Methods

2.1. Study design

The study was a cross-sectional laboratory based evaluation of Aptima assay using whole blood DBS specimens obtained from HIV-exposed neonates. Standards for the Reporting of Diagnostic Accuracy Studies (STARD) were adhered to. The gold standard comparator assay for this study was the CAP/CTM.

2.2. Study setting

The study was conducted at two academic healthcare facilities in Gauteng Province, South Africa; Rahima Moosa Mother and Child Hospital (RMMCH) and Kalafong Provincial Tertiary Hospital (KPTH), between 1st March 2018 and 31st January 2020. These institutions are situated within high HIV prevalence districts, and were sites for a study evaluating the implementation of maternal and infant HIV virological point of care (POC) testing. (Kufa et al., 2020) In the aforementioned study, whole blood samples were collected at time of birth from HIV-exposed neonates and tested concurrently using the CAP/CTM assay in a centralized laboratory.

2.3. Sample collection and preparation

Dried blood spot specimens were prepared from leftover whole blood collected in EDTA tubes. The DBS specimens were prepared

from the collected whole blood samples within one week of the blood draw (stored at 4°C), and spotted on demarcated circles on a pre-perforated DBS Whatman 903 Neonatal STD EU card (GE Healthcare, Westborough, MA, USA) or on Munktel TFN specimen collection cards (Munktel Filter AB, Falun, Sweden). Each DBS circle accommodates approximately 75–80 µl of whole blood. (Rottinghaus et al., 2013) The DBS cards were dried for a minimum of three hours and stored in individual Ziploc bags containing desiccant, and a humidity indicator card. Thereafter the specimens were stored at -80°C. Specimens with enough sample for two spots on a DBS card were eligible for inclusion in the study. In addition to comparing Aptima qualitative HIV results with CAP/CTM results, HIV viral load data from routine laboratory testing of plasma were compared with VL data obtained from testing DBS specimens on the Aptima from the same patient where available.

2.4. Laboratory methods

The Aptima is a dual-target total nucleic acid amplification test for *in vitro* diagnostic use (Rottinghaus et al., 2013) which relies on transcription-mediated amplification (TMA) to amplify the *pol* (polymerase) and *LTR* (long terminal repeat) regions of the HIV genome. The Aptima, which reports both a qualitative and quantitative result (in copies per millilitre without a Ct value), is processed on Hologic's Panther system that allows full automation of molecular testing. Furthermore, the Aptima has been validated for both Munktel TFN and Whatman 903 DBS cards. The diagnostic sensitivity of the Aptima when using DBS specimens is reported by the manufacturer as 99.6%, with a specificity of 99.2%, and a limit of detection (LoD) using DBS specimens of 873 copies/mL. (Hologic Gen Probe, Inc 2018) In addition to a qualitative result, the Panther system reports quantitative VL measurements in copies/mL and log₁₀ copies/mL based on a DBS conversion factor. This conversion factor is designed to prevent overestimation of quantitative measurements which can occur as a result of intracellular nucleic acids when using DBS specimens. Specimens with results listed as "Not Detected" are interpreted as a negative HIV result, while specimens with results listed as "<873 detected" or with a quantified VL within the linear range of the Aptima, indicate HIV was detected i.e., positive HIV result.

Qualitative HIV results obtained from testing performed using venous EDTA whole blood on the CAP/CTM within accredited NHLS laboratories, were used as the gold standard from which test performance of the Aptima assay was calculated. Testing on the CAP/CTM can be performed using either DBS specimens obtained from capillary heel-prick (approximately 60 µl per spot per test) or EDTA anticoagulated whole blood via phlebotomy or arterial puncture (100 µl per test). (Mazanderani et al., 2018) The CAP/CTM is a total nucleic acid dual-target real-time PCR assay targeting the *LTR* and *gag* region of the HIV genome. The LoD for HIV-1 RNA, as determined by the manufacturer using whole blood DBS specimens, is 300 copies/mL. (Roche Molecular Systems, Inc 2013) The reported diagnostic sensitivity of the CAP/CTM is 100%, with a specificity of 99.8%.

HIV viral load data resulted from routine laboratory testing of plasma using the Abbott RealTime HIV-1 Test (Abbott Molecular, Inc., Des Plaines, IL) and the COBAS® HIV-1 Test (Roche Diagnostics, Basel, Switzerland) were compared with VL data from testing DBS specimens on the Aptima. The Abbott test is a real-time quantitative PCR test, targeting the integrase region of the *pol* gene. The LoD is 40 copies/mL and the reported diagnostic specificity of the Abbott test is 100%. (World Health Organization 2018) The Cobas assay is a dual-target real-time quantitative PCR test, targeting the *LTR* and *gag* region of the HIV genome with a LoD of 20 copies/mL. The reported diagnostic specificity of the Cobas assay is 100%. (Roche Molecular Systems, Inc 2013)

2.5. Aptima testing method

Sample preparation: Dried blood spot samples were allowed to thaw for 90 minutes. A single DBS spot was excised and added to the provided specimen aliquot tubes (SAT). Thereafter 1000 μ l of proprietary buffer was pipetted into the SAT and placed on a rocker platform at ambient temperature for 30 minutes. The SAT containing the extracted DBS, was then centrifuged at 3000g for two minutes before being loaded on the Panther system for processing.

Sample processing: All specimens were processed according to the Aptima package insert as per manufacturer's instructions. For the purposes of this study, all specimens that tested positive on the Aptima were repeat tested as follows: the first DBS circle was tested twice using the test order function on the Panther system, a second DBS circle was excised and placed in a separate SAT, and tested once as per manufacturer's instructions. This means that all specimens with an HIV-detected result on the Aptima had three unique test results. Specimens with discordant results, as well as specimens that resulted in an error, were retested by excising an additional DBS and processing according to manufacturer's instructions. The first valid Aptima result was used for evaluation of assay performance.

2.6. Data management and statistical analysis

Date and site of specimen collection, laboratory identifier barcode, hospital number, date of birth, and HIV virological result including available VL data, were collected for each sample. These data were exported into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) for further analysis. The patient data in the database was de-identified prior to analysis. The data were password protected and accessible only to the student, supervisors and designated research staff. Patient study identifiers and laboratory identifier barcode were used as patient identifiers in lieu of patient name, surname or date of birth in order to protect patient confidentiality at time of analysis. Descriptive statistical analysis was performed with median and interquartile ranges used to describe age at DBS testing. A two-by-two table was used to calculate the performance of the Aptima in predicting HIV status amongst infants and young children when using whole blood DBS in comparison to the CAP/CTM. Sensitivity, specificity, PPV, negative predictive value (NPV) and accuracy are reported as proportions with 95% confidence intervals (CI).

Bland-Altman analysis was performed using Stata® 14.2 (Stata-Corp, Texas, USA) to evaluate differences and limits of agreement between VL results on the Aptima compared with Abbott or Cobas results. Bland-Altman analysis was also performed to determine the agreement of VL results obtained from repeat testing using a separate spot from the same DBS card on the Aptima assay. Descriptive statistical analysis was performed with median and interquartile ranges used to describe age at DBS testing as well inter-assay and intra-assay variability of HIV VL $\log_{10}$ values.

2.7. Ethics statement

This study was conducted as per ethical clearance obtained from the University of the Witwatersrand Human Research Ethics Committee, Johannesburg, South Africa (Protocol number M190645). Permission was granted to use stored leftover DBS whole blood specimens from HIV-exposed infants who had EID testing performed as part of a prospective cross-sectional EID point of care implementation study between March 1, 2018 and January 31, 2020 at RMMCH and KPTH (Protocol number M1711115).

3. Results

A total of 378 samples were collected from HIV-exposed infants of which 15 did not meet the inclusion criteria. Of the 363 samples that

met the inclusion criteria, 32 (8.8%) were HIV-detected on CAP/CTM, with 2 of these samples classified as indeterminate as per NHLS verification. There were 331 (91.1%) samples with a negative HIV test result on the CAP/CTM. Fig. 1 provides the study flow with inclusion and exclusion criteria. The median age of patients from which samples were collected from was 1 day (interquartile range [IQR]: 1–2).

Among 32 samples with an HIV-detected result on the CAP/CTM, 30 (93.8%) tested HIV positive on the Aptima. The two samples with an HIV-detected result on CAP/CTM but negative result on the Aptima were both reported as indeterminate on CAP/CTM, as per NHLS verification (Table 1), with Ct values of 33.4 and 36.0. Plasma viral load testing for these two specimens was not performed. Of the 331 samples with an HIV negative result on CAP/CTM, 329 (99.4%) were negative on the Aptima. The two samples with an HIV negative result on CAP/CTM but HIV-detected result on Aptima were both reported as having an HIV VL below the lower limit of quantification (i.e., <873 copies/ml) on initial testing on the Aptima. Both samples yielded the same result i.e., HIV detected but <873 copies/ml, upon replicate testing performed on the same spot. When a separate spot from the same DBS card was used, these samples tested negative for HIV. Compared to the CAP/CTM, the Aptima had a sensitivity of 93.75% (95% CI: 79.19%–99.23%), specificity of 99.4% (95% CI: 97.83%–99.93%), PPV of 93.75% (95% CI: 78.98%–98.36%), NPV of 99.4% (95% CI: 97.73%–99.84%), and overall accuracy of 98.9% (95% CI: 97.2%–99.7%).

There were 37 errors (10.2%) which occurred (Fig. 2) on the Aptima. Twenty-four (64.9%) were due to an internal assay processing error, caused by the low positive control failing. This type of error results in all associated samples in that run being invalid. Twenty-three (95.8%) of these errors were resolved by retesting of specimens. One specimen resulted in an error when retested. The remaining 13 (35.1%) errors that occurred were due to an internal hardware error. In order for this error to be resolved, a hardware update is required. Of these errors, 12 (92.3%) were resolved by retesting of specimens. One sample could not be retested due to an insufficient number of stored DBS spots.

Viral load comparability The Aptima reports a quantitative VL result for all samples that have an HIV-detected result. Among 32 samples with an HIV-detected result on the CAP/CTM, 30 had an HIV-detected result on the Aptima. Of these 30, 20 (62.5%) had routine laboratory VL test data available for comparison from plasma samples taken on the same day as the EID sample. Testing was performed on the Abbott ($n=18$) and the Cobas ($n=2$) assays. The median time between sampling to when the VL was resulted was 1 day (IQR: 0–9) on the Abbott assay and 0 days (IQR: 0–1) on the Cobas assay.

Bland-Altman analysis performed (Fig. 3) showed that the limits of agreement (LoA) between the Aptima and the Cobas assays were wide (LoA [$\log_{10}$ VL]: -2.039 to 2.551) with moderate correlation ($r=0.555$) suggested by Pearson's correlation coefficient. The relative mean difference was 0.256 (CI -0.281 to 0.793). The absolute median log difference between DBS VL measurements on the Aptima, and plasma VL was 0.66 (IQR: 0.36–1.71). Of the 20 samples that had routine laboratory VL test data available, 13 (65%) of these had an absolute difference greater than 0.5 $\log_{10}$ copies/mL.

Repeat testing of samples: Samples with an HIV-detected result on the Aptima were tested an additional two times on the Aptima. One was performed on the same DBS spot as the initial test, using the 'replicate' function on the Aptima, and the other test was performed using a separate DBS spot. Thirty-two samples had an initial HIV-detected result on Aptima. All 32 yielded the same result when repeat testing was performed using the replicate function. However, when testing was performed using a separate spot from the same DBS card, two of the samples tested negative.

In Fig. 4A, results obtained from the first DBS spot tested were compared with results obtained from repeating the first DBS spot (i.e. using the replicate test order function on the Panther system). The Bland-Altman plot (Fig. 4A) showed that the LoA between VL

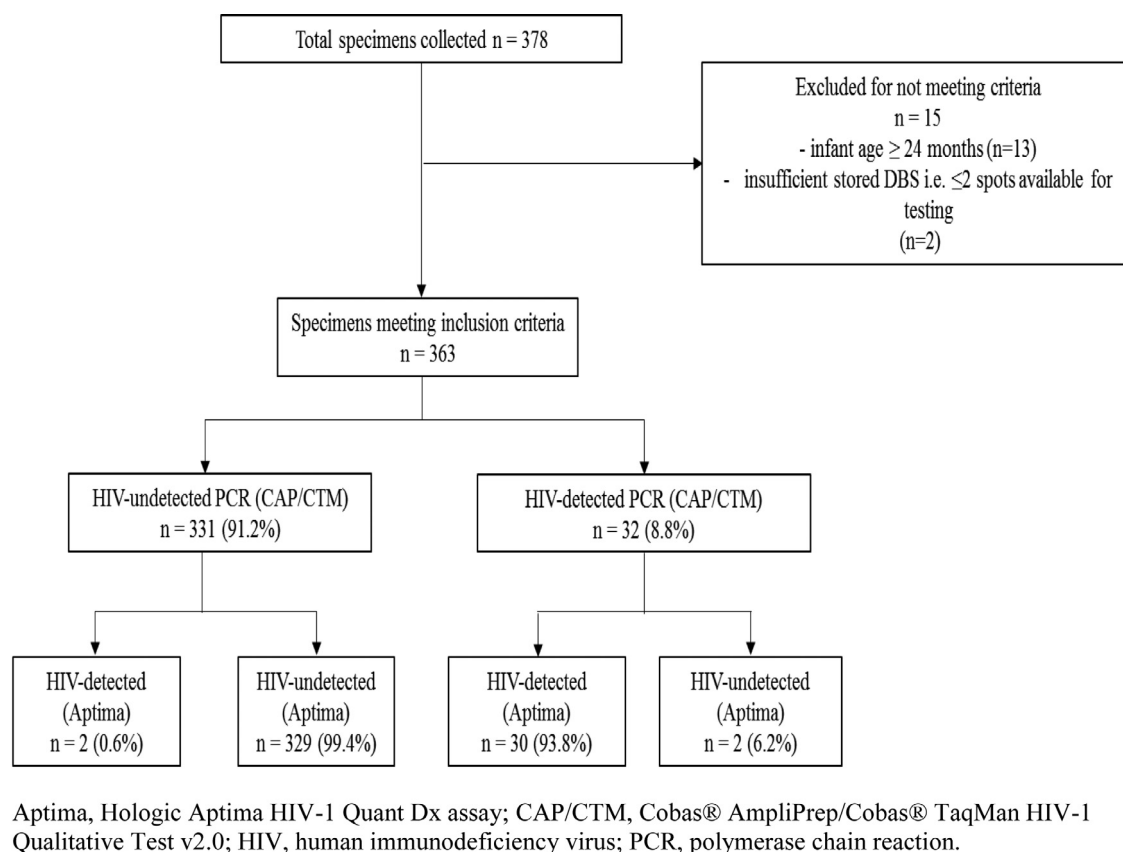


Fig. 1. Study flow with inclusion and exclusion criteria and initial HIV status of specimens.

Aptima = Hologic Aptima HIV-1 Quant Dx assay; CAP/CTM = Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV = human immunodeficiency virus; PCR = polymerase chain reaction.

measurements obtained on initial testing, and VL measurements obtained from the repeated result of the same DBS sample showed variance ($LoA [log_{10}VL] = -1.011$ to 0.761). However, Pitman's Test showed that the variance in VL measurements obtained when using the replicate function on the Aptima ($P = 0.529$) was not significant. The absolute median log_{10} difference between VL measurements obtained from initial testing on the Aptima and repeat testing was 0.286 (IQR: 0.176 – 0.4).

In Fig. 4B, results obtained from the first DBS spot tested were compared with results obtained from testing a second DBS spot from the same DBS card at a different time-point. The Bland-Altman plot showed that the LoA between the VL measurements was wide ($LoA [log_{10}VL] = -0.801$ to 0.503). However, Pitman's Test showed that there was no significant variance in VL measurements obtained from initial testing, and subsequent testing performed on a different DBS spot

from the same DBS sample ($P = 0.674$). The absolute median log_{10} difference between VL measurements was 0.155 (IQR: 0.04 – 0.36).

4. Discussion

While previous studies have evaluated the performance of the Aptima using DBS among infants, (Mwau et al., 2020, Sahoo et al., 2016) we evaluated the Aptima within a defined population of HIV-exposed neonates. Furthermore, in this study the accuracy of 'replicate' function of the Aptima assay was also evaluated. The results demonstrate good diagnostic performance of the Aptima in comparison with CAP/CTM as a qualitative EID assay, and support the use of the Aptima as either an EID screening assay or to confirm HIV-detection in samples that have yielded an HIV-detected result on an alternate assay.

In addition to the diagnostic performance of the Aptima, we also found good reliability of Aptima HIV VL results. In general, VL performed on DBS specimens yielded higher results than VL performed on plasma with poor inter-assay reliability, likely due to the presence of intracellular viral DNA and RNA, despite the Aptima assay applying a DBS conversion factor designed to prevent overestimation of quantitative viral load measurements.

One of the challenges in this study was the high error rate experienced. We experienced two types of errors, namely internal assay processing errors and hardware errors. Although, the internal assay processing errors were quick to resolve by repeat testing on the Aptima, as all specimens associated with that run had invalid results these errors proved to be numerous and costly. Alternately, the hardware errors only occurred in selected SATs where the Panther system was unable to detect DBS specimen in the tube position on the testing rack. We were able to obtain Aptima test results for these specimens using the

Table 1
HIV results using the Aptima versus CAP/CTM assay.

HIV status	CAP/CTM screening test result as per NHLS verification	Aptima test result
HIV positive (n=32)	PCR positive (n=30)	HIV- detected (n=30)
	PCR indeterminate (n=2)	HIV-undetected (n=2)
HIV negative (n=331)	PCR positive (n=0)	HIV-detected (n=0)
		HIV-undetected (n=0)
	PCR negative (n=331)	HIV-detected (n=2)
		HIV-undetected (n=329)

Aptima = Aptima HIV-1 Quant Dx assay; CAP/CTM = Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV = human immunodeficiency virus; NHLS = National Health Laboratory Service; PCR = polymerase chain reaction.

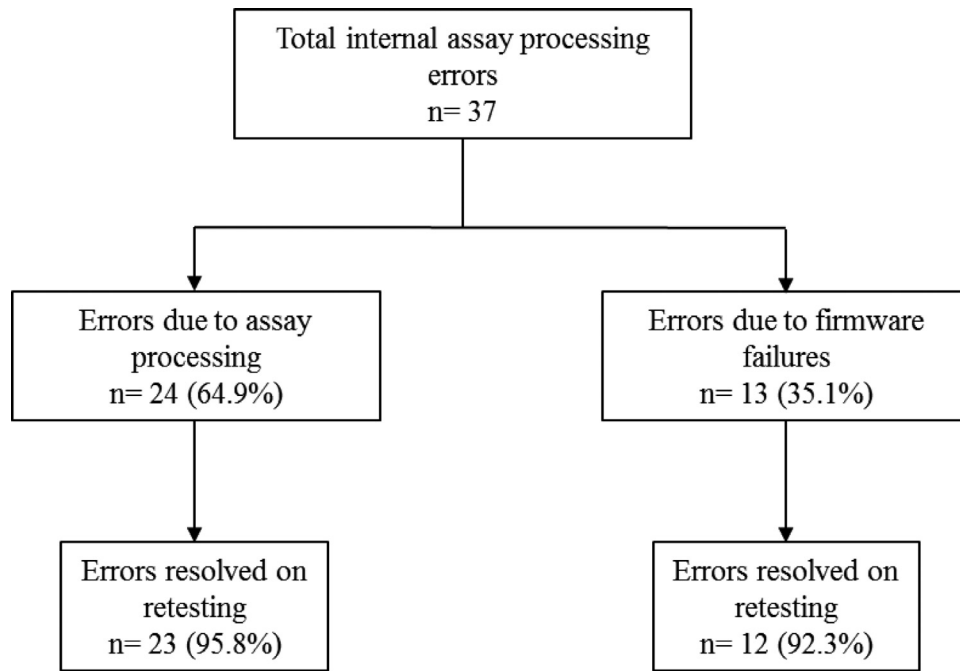


Fig. 2. Internal assay processing errors on the Aptima.

'replicate' function (i.e., additional DBS spot was not required). However, it is worth noting that the assistance of a technician was required to resume testing. In this study we excluded one specimen due to the fact that all DBS spots for this specimen were depleted on account of the errors experienced. In addition to this, repeat testing, including using the replicate function, requires additional reagents as well as consumables involved in processing of specimens. The errors that occurred consumed the equivalent of one Aptima test kit (i.e., 100 tests). This meant that an additional kit had to be purchased to accommodate testing of all collected eligible specimens in this study.

A number of important limitations need to be considered regarding these findings. The clinical specimens used in this study were

restricted to HIV-exposed neonates from only two facilities. Hence, results may not be generalizable to the overall EID program. However, considering VL results as well as positivity rates at birth have been found to be lower among neonates compared with older infants, thereby potentially impacting the sensitivity and PPV of an assay, it is likely that Aptima will perform better within the overall HIV infant diagnosis program compared with just birth testing. Additionally, as DBS specimens were tested on Aptima and EDTA whole blood specimens tested on CAP/CTM, and as the DBS specimens were prepared from the whole blood EDTA samples within one week of the blood draw, there may have been the opportunity for cell lysis as well as RNA degradation thereby potentially compromising the accuracy of

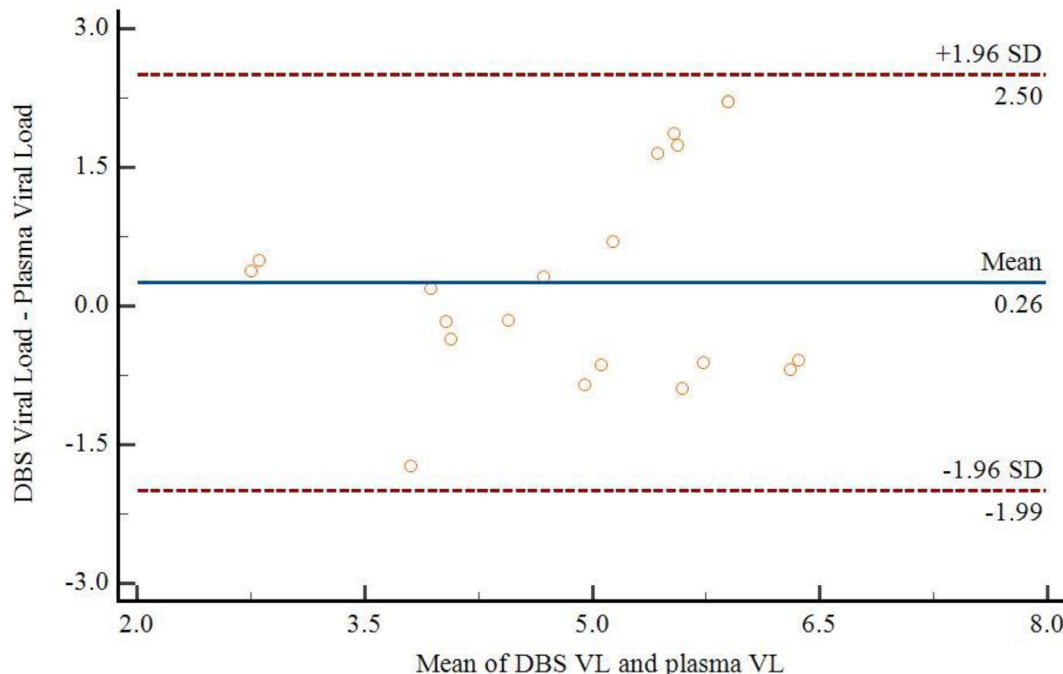
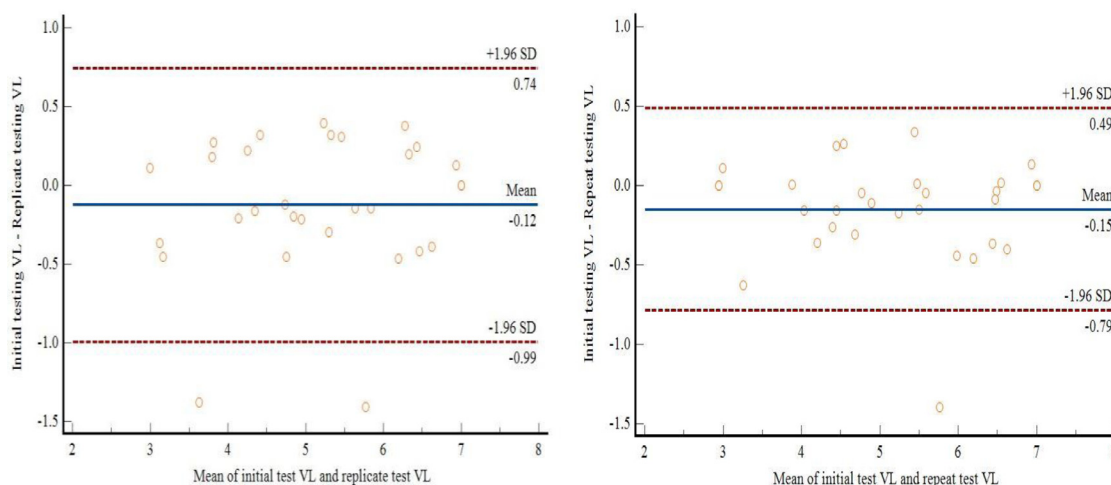


Fig. 3. Bland-Altman plot of agreement between viral load DBS measurements on Aptima and plasma viral load.



a: Bland-Altman plot of agreement. Comparison of VL measurements obtained on the Aptima when using the 'replicate' function on a single spot from the DBS card

b: Bland-Altman plot of agreement. Comparison of initial VL results on the Aptima, compared to VL results obtained from testing a separate spot from the same DBS sample

Fig. 4. Bland-Altman plot of agreement of viral load measurements performed using DBS on the Aptima assay

A: Bland-Altman plot of agreement. Comparison of VL measurements obtained on the Aptima when using the 'replicate' function on a single spot from the DBS card

B: Bland-Altman plot of agreement. Comparison of initial VL results on the Aptima, compared to VL results obtained from testing a separate spot from the same DBS sample.

both qualitative and quantitative Aptima results. (Hardie et al., 2019) Despite these limitations, Aptima performed well as an EID assay with only four discordant EID results between the Aptima and the CAP/CTM assays. Two infants with a negative HIV result on the CAP/CTM had HIV-detected results on the Aptima, both of which were below the lower limit of quantification and both tested negative on repeat testing. Furthermore, two infants with an HIV-detected result on the CAP/CTM tested negative on Aptima, both of which were reported as indeterminate as per NHLS verification practices. Unfortunately, we were unable to ascertain whether these four infants were true positives or false positives as no follow-up test results were retrievable. As such, we cannot conclude whether performance of the Aptima assay would be better than the CAP/CTM for resolving diagnostic dilemmas such as indeterminate results. However, our findings suggest that HIV-detected results below the limit of quantification (i.e., <873 copies/ml) on Aptima should be interpreted with caution, as well as irreproducible HIV-detected results, in keeping with findings on CAP/CTM assay. (Mazanderani et al., 2018) Regarding accuracy of VL results on the Aptima, our findings suggest DBS specimens will likely yield significant over quantification of VL compared with plasma specimens. However, on account of our small sample size of eligible specimens we are unable to conclusively determine whether DBS specimens can be used for HIV VL monitoring.

5. Conclusion

The diagnostic performance of the Aptima EID assay as a qualitative test was comparable to that of the CAP/CTM. Future studies should explore the integration of the Aptima within the current EID testing algorithm and how it can be utilized to resolve diagnostic dilemmas particularly in infants exposed to ARV drugs.

Credit author statement

Aur lie Mukendi, Gayle Sherman, and Ahmad Haeri Mazanderani conceptualized the study. Aur lie Mukendi, performed the data

collection. Aur lie Mukendi, and Tendesayi Kufa performed data analysis. Aur lie Mukendi wrote the first draft of the manuscript. All authors reviewed the manuscript and approved the final version for submission.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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