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Field evaluation of Abbott Real Time HIV-1 Qualitative test for early infant diagnosis using dried blood spots samples in comparison to Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Qual Test in Kenya

Joy Chang[a,*], **Kenneth Omuomo**[b], **Emily Anyango**[b], **Leonard Kingwara**[b], **Frank Basiye**[c], **Alex Morwabe**[b], **Vedapuri Shanmugam**[a], **Shon Nguyen**[a], **Jennifer Sabatier**[a], **Clement Zeh**[b], and **Dennis Ellenberger**[a]

[a]Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

[b]Kenya Medical Research Institute (CDC/KEMRI), Kisumu, Kenya

[c]Centers for Disease Control and Prevention (CDC), Kenya

Abstract

Timely diagnosis and treatment of infants infected with HIV are critical for reducing infant mortality. High-throughput automated diagnostic tests like Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Qual Test (Roche CAPCTM Qual) and the Abbott Real Time HIV-1 Qualitative (Abbott Qualitative) can be used to rapidly expand early infant diagnosis testing services. In this study, the performance characteristics of the Abbott Qualitative were evaluated using two hundred dried blood spots (DBS) samples (100 HIV-1 positive and 100 HIV-1 negative) collected from infants attending the antenatal facilities in Kisumu, Kenya. The Abbott Qualitative results were compared to the diagnostic testing completed using the Roche CAPCTM Qual in Kenya. The sensitivity and specificity of the Abbott Qualitative were 99.0% (95% CI: 95.0–100.0) and 100.0% (95% CI: 96.0–100.0), respectively, and the overall reproducibility was 98.0% (95% CI: 86.0–100.0). The limits of detection for the Abbott Qualitative and Roche CAPCTM Qual were 56.5 and 6.9 copies/mL at 95% CIs ($p = 0.005$), respectively. The study findings demonstrate that the Abbott Qualitative test is a practical option for timely diagnosis of HIV in infants.

Keywords

HIV; Early infant diagnosis; Dried blood spots

[*]Corresponding author at: International Laboratory Branch (ILB), Division of Global HIV/AIDS (DGHA), Center of Global Health (CGH), Centers for Disease Control and Prevention (CDC), 1600 Clifton Road, NE, Atlanta, GA 30333, USA. Tel.: +1 404 639 1589. ckc7@cdc.gov (J. Chang).

1. Introduction

An estimated 3.4 million children were living with HIV at the end of 2011 and 91% of these children reside in Sub-Saharan Africa (WHO HIV/AIDS Topics, 2012). Greater than 90% of the annual pediatric HIV infections globally are acquired vertically through mother-to-child transmission (Global Report. UNAIDS Report on the Global AIDS Epidemic, 2010). While the number of children receiving antiretroviral therapy (ART) has increased from approximately 456,000 in 2010 to 562,000 in 2011, the pediatric ART coverage rate is only 28% (WHO HIV/AIDS Topics, 2012). This low coverage rate is further affected by the fact that an estimated 390,000 children were newly infected with HIV in 2010 (WHO Progress report, 2011) and the mortality of infants infected with HIV is quite high in the first year (Newell et al., 2004; Marston et al., 2011).

Early infant diagnosis is an important step to link infants infected with HIV to ART and to reduce infant morbidity and mortality (Aledort et al., 2006; Stevens et al., 2008b). However, the lack of access to virologic testing remains a challenge. The use of dried blood spots (DBS) has facilitated detection of HIV in settings with limited resources (Sherman et al., 2005). The test most widely used in the public health sector is the Roche Amplicor HIV-1 DNA Test, version 1.5, a manual DNA PCR test (Nelson et al., 2011). However, this Roche Amplicor DNA PCR test requires 8 h and more than 40 labor-intensive steps to obtain results for 96 samples (Amplicor HIV-1 DNA Test, version 1.5 package insert, Roche Diagnostics, 2006). Such a test presents great challenges for laboratories to keep pace with the increased volume of samples and to ensure quality testing results.

In order to meet the rising demand for HIV viral load testing, Roche Diagnostics developed the Roche CAPCTM HIV-1 test in 2007, an automated high-throughput test for HIV viral load testing. Later in the same year, Roche developed another assay, the Roche CAPCTM Qual to meet the need of early infant diagnosis of HIV. The Roche CAPCTM Qual has been evaluated for early infant diagnosis by several laboratories (Schumacher et al., 2007; Stevens et al., 2008a; Jani et al., 2012) and being used in many countries where a large number of DBS samples are received routinely (Schumacher et al., 2007; Stevens et al., 2008a; Jani et al., 2012; Kageha et al., 2012; Maritz et al., 2012).

In 2010, the collaboration between Abbott Molecular and Clin-ton Health Access Initiative resulted in the development of the Abbott Qualitative test for early infant diagnosis using a high-throughput instrument, Abbott m2000 (Abbott HIV Test Kits to Clinton Health Access Initiative, 2010). In 2011, the Abbott Qualitative received the CE Mark (*Conformité Européenne* or European Conformity); the assay produced highly accurate results from both DBS and plasma samples (Abbott Press Release, 2011). This was an important advance to expanding early infant diagnosis testing capacity in countries with increasing testing volumes since a new higher throughput assay provided the countries with a new technical design, potential price competition, and greater access to technical support. When a new diagnostic test is developed, it is critical to validate its performance characteristics in the environment in which it will be used and compare its performance characteristics with the existing test. The Abbott Qualitative was previously compared to the Roche Amplicor HIV-1 DNA Test, version 1.5, a widely used assay for early infant diagnosis (Nelson et al.,

2011). In Nelson's study, the Abbott Qualitative demonstrated good specificity but poor sensitivity when compared to the Roche Amplicor HIV-1 DNA Test, version 1.5. However, Nelson et al. suggested that since infants infected with HIV usually have very high HIV-1 RNA levels, greater than 1000 copies/mL, the Abbott Qualitative may still be a good alternative to the Roche Amplicor HIV-1 DNA Test, version 1.5 for the laboratories with the rising demands for early infant diagnosis. In this study, the performance characteristics of the Abbott Qualitative were evaluated and compared with the performance characteristics of the fully automated Roche CAPCTM Qual for early infant diagnosis.

2. Materials and methods

2.1. Sample source and storage

The clinical samples were DBS collected from 100 HIV-positive and 100 HIV-negative infants with an age range between 6 weeks to 18 months. These DBS were previously tested using the Roche CAPCTM Qual as part of the routine early infant diagnosis testing services at the reference laboratory of Kenya Medical Research Institute/Centers for Disease Control and Prevention (CDC), Kisumu, Kenya. All the HIV-positive DBS were confirmed using the Kenya early infant diagnosis testing algorithm. To protect the patients' privacy, identification and medical information of the infants were delinked from the patients' testing results before the testing results were sent to the study investigators at CDC, Atlanta, for data analysis. The DBS were stored at −20°C prior to testing in the study.

2.2. Roche CAPCTM Qual

The Roche CAPCTM Qual (Roche Diagnostics, Johannesburg, South Africa) includes three major steps (COBAS Ampliprep/COBAS Taqman HIV-1 Qual Test package insert, Roche Diagnostics, 2010): (1) DBS sample pre-extraction, (2) automated nucleic acid extraction using a genetic silica-based capture technique, and (3) reverse transcription and PCR amplification. Briefly, one 12 mm diameter DBS from each patient was excised from the Whatman filter paper and placed into a sample tube (S-tube) and incubated with 1100 μl of sample pre-extraction (SPEX) buffer at 56 °C for 10 min in a thermo-mixer vortex at 1000 rpm. After the incubation step, the S-tubes containing SPEX buffer and DBS eluent were processed on the Roche COBAS AmpliPrep (CAP) instrument along with the testing kit's positive and negative controls. HIV-positive and HIV-negative DBS quality control samples produced by the CDC International External Quality Assessment Program (CDC EQA Program) (Garcia et al., 2014) were also run concurrently with the patient samples to monitor the DBS testing process. The reverse transcription, PCR amplification, and detection of PCR products were performed by the Roche COBAS TaqMan (CTM) instrument. After PCR amplification, if the Roche kit controls and the internal control (IC) of each sample were valid then the instrument's software, AmpliLink, analyzed the data and provided a qualitative result for each patient sample. The Roche CAPCTM Qual was validated at the laboratory of the Kenya Medical Research Institute/CDC, Kisumu, Kenya before it was used for the routine early infant diagnosis testing.

2.3. Abbott Qualitative

Abbott Qualitative test (Abbott Molecular, Wiesbaden, Germany) also includes three major steps (Abbott Real Time HIV-1 Qualitative package insert, Abbott Molecular, 2010): (1) pre-extraction, (2) automated nucleic acid extraction on the Abbott m2000sp (Abbott m2000 sample processing), and (3) reverse transcription and PCR amplification on the Abbott m2000rt (Abbott m2000 Real Time). Briefly, two 12 mm diameter DBS were excised from the Whatman filter paper and placed into a 15 mL falcon tube and incubated with 1.7 mL of mLysis buffer at room temperature for 20 min. Prior to the automated nucleic acid extraction on the Abbott m2000sp, the liquid in each sample tube was transferred to a reaction vessel and the DBS was discarded. The Abbott m2000sp then performed the nucleic acid extraction, washing, and elution. The internal control (IC) was introduced in the mLysis buffer before the mLysis buffer was loaded on the Abbott m2000sp. HIV positive and negative DBS quality control samples from the CDC EQA Program were also included in each run to monitor the DBS preparation, nucleic acid extraction, and amplification processes. After the sample extraction was completed, the amplification reagents and the master mix tube were loaded on the Abbott m2000sp. The master mix was then added to the 96-well Optical Reaction Plate with the extracted nucleic acids on the Abbott m2000sp. For the Real-time PCR amplification, the 96-well Optical Reaction Plate was sealed and transferred to the Abbott m2000rt. When the amplification was completed, if the Abbott kit controls and IC were valid then the software file on the Abbott m2000rt analyzed the Real-time PCR data and assigned a qualitative result to each sample.

To analyze the reproducibility for the Abbott Qualitative, only one 12 mm DBS was used per test because, at the time when the reproducibility study was performed, Abbott Molecular received a WHO certificate for the Abbott Qualitative test to use one DBS rather than 2 DBS per test. The 80 samples (40 HIV-1 positive and 40 negative samples) were selected from the 200 samples that had at least three 12 mm DBS remaining.

2.4. Limits of detection (LOD) of Abbott Qualitative and Roche CAPCTM Qual

The DBS used for the LOD study were prepared using HIV-negative ethylenediaminetetraacetic acid (EDTA) whole blood (Tennessee Blood Services, Memphis, TN) spiked with the 8E5 cells (Folks et al., 1986; Gendelman et al., 1987) at the concentrations of 100, 80, 40, 20, 10, 5, 2.5, 1.25, and 0 cells/mL. Because each 8E5 cell contains one copy of HIV-1 genome, the copies/mL concentration designation was used. Each concentration was tested ten times on both the Abbott Qualitative and the Roche CAPCTM Qual tests.

2.5. Statistical analysis

The 95% confidence interval (CI) was used to analyze the test's sensitivity and specificity. The reproducibility of the Abbott Qualitative was analyzed by Fleiss Kappa Statistics (Fleiss, 1971) using SAS, version 9.3. The LODs were analyzed using probit analysis with the 95% CI using SAS, version 9.3.

3. Results

3.1. Sensitivity and specificity of Abbott Qualitative

In this study, 100 HIV-positive and 100 HIV-negative DBS were used to calculate the sensitivity, specificity, and 95% confidence interval for the Abbott Qualitative. Of the 100 DBS that were tested HIV-positive by the Roche CAPCTM Qual, 99 were found to be HIV-positive by the Abbott Qualitative (Table 1). The one negative sample was tested twice by the Abbott Qualitative and the test result did not change, resulting in sensitivity of 99.0% (95% CI: 94.6–100.0). Interestingly, the Cycle Threshold (CT) value for the discordant DBS was 26 as measured by the Roche CAPCTM Qual, which indicates a strong signal and an adequate amount of input target DNA in this sample. All 100 DBS that tested negative by the Roche CAPCTM Qual also tested negative by the Abbott Qualitative, yielding specificity of 100.0% (95% CI: 96.4–100.0). The overall agreement between the two tests was 99.5%.

3.2. Reproducibility of Abbott Qualitative

To examine the reproducibility of the Abbott Qualitative, 80 DBS were tested in triplicate. Only one positive sample failed during the nucleic acid extraction phase on two separate testing runs. Therefore, the overall reproducibility of Abbott Qualitative was 0.98 (95% CI: 0.86–1.00) (Table 2).

3.3. LODs of Abbott Qualitative and Roche CAPCTM Qual

The LODs for the Abbott Qualitative and the Roche CAPCTM Qual tests are shown in Table 3. A probit analysis showed that the LOD of Abbott Qualitative was 56. 48 copies/mL (95% CI: 42.06–96.55 copies/mL) (Fig. 1) and the LOD of Roche CAPCTM Qual was 6.91 copies/mL (95% CI: 5.09–13.88 copies/mL) (Fig. 2).

4. Discussion

Because of the tremendous effort invested to improve early infant diagnosis coverage, hundreds and thousands of infant DBS will need to be tested each month at many regional and national laboratories, similar to the reference laboratory at the Kenya Medical Research Institute. Higher throughput and automated testing will enable these clinical laboratories to increase their testing capacities and return the diagnostic testing results back to the clinicians in a shorter period of time. In addition, automated testing could minimize the result variability among different test operators, alleviate the qualified medical laboratory staff shortages, and reduce the human errors, which often occur during the manual testing and data transcription processes (Hölzl et al., 2003). In this study, the performance characteristics of the automated Abbott Qualitative were evaluated and the results suggested that this test is a feasible option to perform early infant diagnosis of HIV in a reliable and timely fashion.

4.1. Sensitivity and specificity of Abbott Qualitative

The Abbott Qualitative demonstrated excellent sensitivity of 99.0%; a single HIV-positive sample tested by the Roche CAPCTM Qual was determined to be HIV-negative twice by the

Abbott Qualitative. The discordant HIV-negative result by the Abbott Qualitative test appears not to be due to a limited DNA concentration issue, which is supported by the fact that other DBS with CT values of 26 by the Roche CAPCTM Qual were all detected by the Abbott Qualitative. One explanation for the discordant result could be the loss of the input DNA during the nucleic acids extraction process on the Abbott m2000sp. However, it is difficult to explain how the nucleic acids of this one sample were lost in two separate extractions. Another possible explanation for the discordant result could be differences in the primer/probe designs of the Roche CAPCTM Qual and the Abbott Qualitative tests. According to the Roche package insert, the Roche CAPCTM Qual targets the *gag* region of HIV-1 genome, whereas the Abbott Qualitative targets the *pol* integrase region.

As for the specificity, the Abbott Qualitative demonstrated excellent specificity of 100.0% (Table 1). These findings are consistent with Nelson et al., which is the only other published evaluation of Abbott Qualitative, where they compared the Abbott Qualitative with the Roche Amplicor and also reported 100% specificity (Nelson et al., 2011).

It should be noted that all of the 200 infant DBS samples were tested using one DBS per test by the Roche CAPCTM Qual and two DBS per test on the Abbott Qualitative at the time when the sensitivity, specificity, and agreement were evaluated. Since each patient had only five blood spots per DBS card, there were not enough DBS remaining to repeat the sensitivity, specificity, and agreement experiments using one DBS per test for the Abbott Qualitative. Nevertheless, the Abbott Qualitative test demonstrated excellent specificity of 100.0% (Table 1) and a very good agreement (99.5%) with the Roche CAPCTM Qual. It is possible that the sensitivity of the Abbott Qualitative may be lower than 99.0% and the agreement between the Roche CAPCTM Qual and the Abbott Qualitative tests may not be as good as 99.5% if only one DBS is used per test since the DNA input concentration would be less. However, the reproducibility data suggested that if one DBS was used per test then the sensitivity and agreement of the Abbott Real Time HIV-1 Qualitative test might not be different.

4.2. Reproducibility of Abbott Real Time HIV-1 Qualitative

As mentioned previously, one DBS per test was used in the reproducibility study. Of the 80 samples tested in triplicate, only one sample failed to produce consistent results, which resulted in an overall reproducibility of 0.98. The DBS that was not consistent had one positive and two failed results. The positive result showed a CT value at 25.4 indicating an adequate amount of target DNA input. The two failed results were associated with two error codes, 3110 and 4447. The 3110 error code indicated that there was either an obstruction or no liquid was found in the sample tube, which could be due to either air bubbles or an obstruction in the sample tube during the sample aspiration. The 3110 error code can be avoided by gently tabbing the sample tubes to break the bubbles before loading the sample tubes on the Abbott m2000sp. The 4447 error code indicated that there was either a pipetting error in the master-mix preparation or the plate preparation on the Abbott m2000sp, which are probably caused by bubbles in the system's tubing or an instrument liquid handler error. To avoid bubbles in the system tubing, extensive system priming before running the samples may reduce the chances of having the same problem.

4.3. LODs of Abbott Qualitative and Roche CAPCTM Qual

The goal of the LOD experiment was to directly compare the potential differences between the Abbott Qualitative and Roche CAPCTM Qual tests. The probit analyses indicated that the LODs were 56.48 copies/mL for the Abbott Qualitative and 6.9 copies/mL for the Roche CAPCTM Qual. Although there was a difference between the two LODs, the difference may not have a significant impact on early infant diagnosis since the LODs of both the Abbott Qualitative and the Roche CAPCTM Qual tests were considered low compared to the high viral loads of the newly infected infants (Nelson et al., 2011; Shearer et al., 1997).

The Abbott Qualitative LOD in this study appears to be lower than the LOD claimed by Abbott Molecular (2500 copies/mL using two DBS per test) (Real Time HIV-1 Qualitative, 2012). However, it does not necessarily imply an LOD improvement because different sources of input nucleic acids were used in the two determinations. In Abbott Molecular's LOD determination, an RNA-based HIV viral standard from the Virology Quality Assurance Laboratory of the AIDS Clinical Trial Group was used. Alternatively, in this study, 8E5 cells, a DNA-based material, which is primarily composed of HIV proviral DNA, were used. The LOD information for the Roche CAPCTM Qual is not available to the end user. The 8E5 cells have been used as quality control standards in HIV testing (Burgard et al., 2000; Jani et al., 2012; Garcia et al., 2014) and in a DBS collection filter paper study for HIV-1 DNA PCR (Masciotra et al., 2012). However, no other information was found in the literature about using the 8E5 cells for the LOD determination of other diagnostic assays for early infant detection of HIV. The advantage of using 8E5 cells for LOD determination is that each 8E5 cell contains one copy of the viral genome. The disadvantage is that 8E5 cells represent only one HIV-1 subtype (B) and other 8E5-like HIV-1 subtypes do not exist, which could possibly represent the multitude of subtypes found in Africa. Other non-B HIV subtypes could possibly affect the LOD for the Abbott Qualitative but the differences would not be expected to be major since the Abbott Qualitative test is targeting the pol region of HIV-1, which is a fairly conserved region (Tebit et al., 2009). The testing results in this study also support the Abbott Qualitative test's ability to detect other subtypes since the clinical samples were primarily HIV subtypes A and D, but a few other subtypes and recombinant viruses have been found within the recent and long-term infected populations in western Kenya (data not published).

4.4. Differences between Abbott Qualitative and Roche CAPCTM Qual

Unlike the Roche CAPCTM Qual, a closed system test, the Abbott Qualitative was designed to be run on an open system. The Abbott Qualitative also requires more manual manipulations by the laboratory worker before the samples are loaded onto the Abbott m2000sp and m2000rt than the Roche CAPCTM Qual. For example, the Abbott Qualitative requires test operators to remove the DBS from the sample tubes before the sample tubes are loaded on the instrument, which is not required when using the Roche CAPCTM Qual. Because the Abbott Qualitative is an open system test, the test could be more prone to the sample cross-contamination if operators do not follow the procedures. However, the Abbott Qualitative test provides a break point between the nucleic acid extraction and the target amplification procedures, which is not provided by the Roche CAPCTM Qual. This break point enables operators to save the extracted nucleic acids and proceed to Real Time

amplification later if there is a power outage at this point and also provides the operators with flexibility to conduct other molecular work such as sequencing for drug resistance testing when needed.

The Abbott m2000 instrument can process 96 samples within 8 h, which is the same as the Roche CAPCTM 96 instrument, if an appropriate number of personnel and reagents are available. This means that a laboratory can theoretically test approximately 2000 infant DBS monthly. Based on U.S. pricing information, the Abbott m2000 instrument costs approximately $160,000 and the Roche CAPCTM 96 instrument costs $200,000, and the price of the Abbott Qualitative test is around $37 per test which is about $10 higher than the price of Roche CAPCTM Qual. However, the instrument and reagents costs in resource-limited settings are likely to be different from the quoted U.S. costs. Furthermore, there are many other factors that may influence costs such as consortium procurement and negotiated volume-based reductions, instrument rental agreements and public–private partnership initiatives with industry.

5. Conclusions

This study demonstrated a significant and reliable agreement between the Abbott Qualitative and the Roche CAPCTM Qual tests, two automated qualitative tests for early infant diagnosis. With the global plan launched in 2011 to eliminate new HIV infections among children by 2015 (Global Plan Towards the Elimination of New Infections Among Children by 2015 and Keeping Their Mothers Alive, 2011), more pregnant women have been and will be screened for HIV and more infants exposed to HIV will be tested for HIV infection. As another high-throughput test, the Abbott Qualitative test will help countries supported by the US President’s Emergency Plan for AIDS Relief Plan to scale up the early infant diagnosis testing capacity and to reach the AIDS-Free generation goal.

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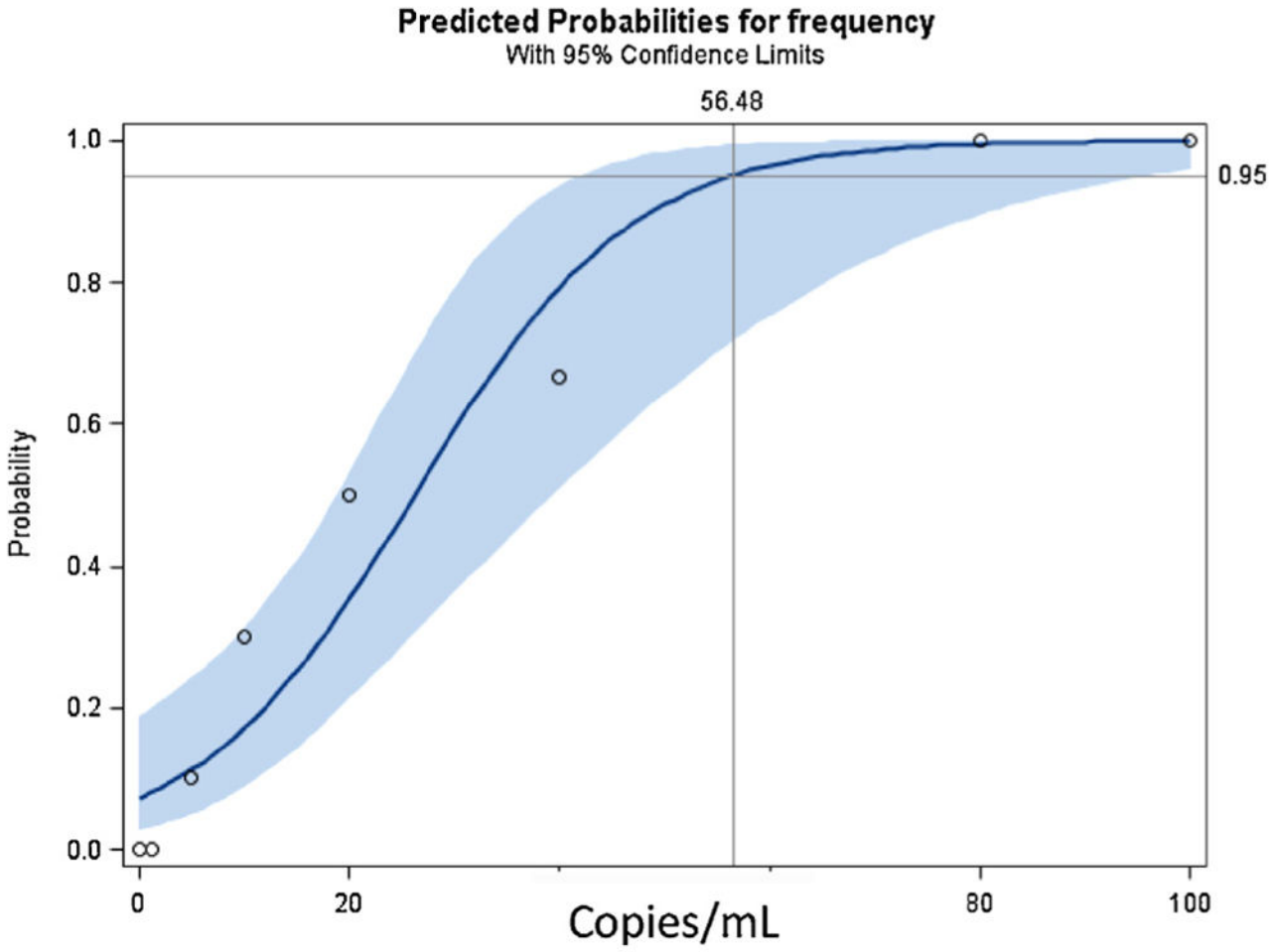

Fig. 1.

Predicted probability of detecting HIV-1 at a given concentration of 8E5 cells for Abbott Qualitative. The relationship between the proportion of number of replicates from each dilution in which HIV was detected and the corresponding concentration of 8E5 cells was used to predict the lowest concentration of 8E5 cells at which 95% of samples would be positive for the presence of HIV. This regression analysis calculation was performed using the probit procedure in SAS, version 9.3.

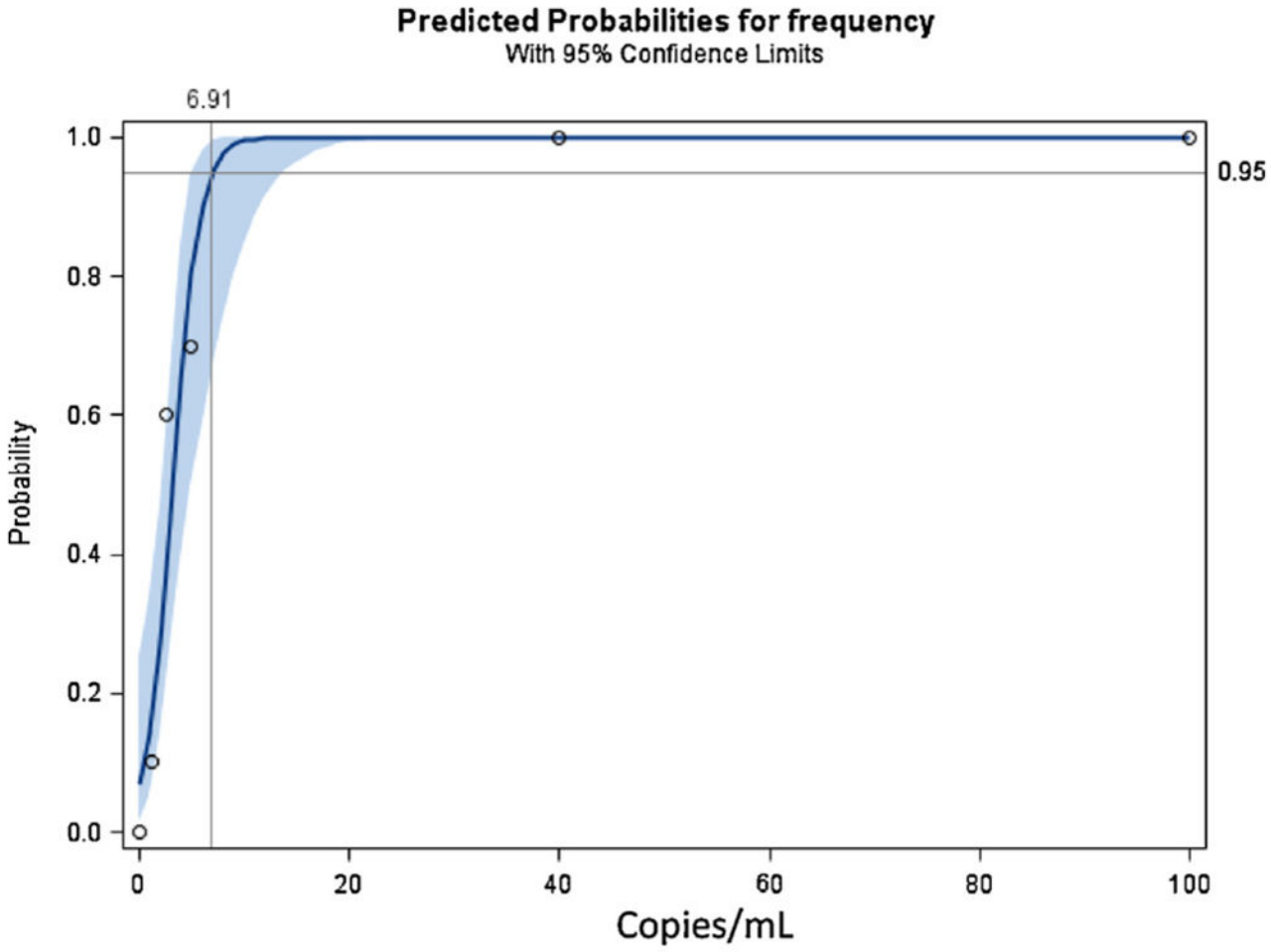

Fig. 2.

Predicted probability of detecting HIV-1 at a given concentration of 8E5 cells for Roche CAPCTM Qual. The relationship between the proportion of number of replicates from each dilution in which HIV was detected and the corresponding concentration of 8E5 cells was used to predict the lowest concentration of 8E5 cells at which 95% of samples would be positive for the presence of HIV. This regression analysis calculation was performed using the probit procedure in SAS, version 9.3.

Table 1

Summary of testing results by Abbott Qualitative and Roche CAPCTM Qual.

		Roche			Sensitivity (95% CI[a])	Specificity (95% CI[a])
		HIV +	HIV −	Total		
Abbott	HIV +	99	0	99	99.0% (94.6%, 100.0%)	100.0% (96.4%, 100.0%)
	HIV −	1	100	101		
	Total	100	100	*N* = 200		

[a]CI, confidence interval.

Table 2

Reproducibility of Abbott Qualitative.

Results	Kappa	95% CI[a]	
Failed	0.5	0.4	0.62
Negative	1	0.87	1
Positive	0.98	0.88	1
Overall	0.98	0.86	1

[a] CI, confidence interval.

Table 3

Limits of detection of Abbott Qualitative and Roche CAPCTM Qual.

Concentration (copies/mL)[a]	Roche			Abbott		
	# of samples	Detection rate (%)[b]	LOD probit[c]	# of samples	Detection rate (%)[b]	LOD probit[c]
0	10	0	0.07	10	0	0.07
1.25	10	10	0.17	10	0	0.08
2.5	10	60	0.36	NA	NA	NA
5	10	70	0.8	10	10	0.11
10	10	100	1.00	10	30	0.17
20	NA	NA	NA	10	50	0.35
40	10	100	1.00	9	67	0.79
80	NA	NA	NA	10	100	0.99
100	10	100	1.00	10	100	1.00

[a] HIV-1 genome copy concentrations.

[b] The percentage of samples or replicates that were detected at different 8E5 cell concentrations.

[c] Probit analysis projected detection rate at different 8E5 cells concentrations.

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