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Early Diagnosis of HIV-1 and HIV-2 Using Cobas HIV-1/HIV-2 Qualitative Test: A Novel Qualitative Nucleic Acid Amplification Test for Plasma, Serum, and Dried Blood Spot Specimens

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Background: Nucleic acid amplification tests (NATs) minimize the time from HIV infection to diagnosis, reducing transmission during acute HIV. NATs are especially useful for diagnosing HIV in children younger than 18 months and discriminating between HIV-1 and HIV-2.

Methods: We evaluated the performance of the cobas HIV-1/HIV-2 qualitative (cobas HIV-1/2 Qual) test for use on cobas 6800/8800 Systems. The results of adult plasma and serum samples and pediatric dried blood spots were compared with those of the recomLine HIV-1 & HIV-2 Immunoglobulin G serological test and COBAS AmpliPrep/COBAS TaqMan HIV-1 qualitative test, v2.0. Genotype inclusivity and limits of detection were determined, and sensitivity on seroconversion panels was compared with that in the Bio-Rad Geenius HIV 1/2 Confirmatory Assay, Abbott ARCHITECT HIV Ag/Ab Combo serological test, and cobas TaqScreen MPX, v2.0.

Results: Concordance of cobas HIV-1/2 Qual test with the comparator serological test and COBAS AmpliPrep/COBAS TaqMan test was ≥99.6% with all sample types. Reactivity with all HIV genotypes was 100%. LOD in plasma samples was 14.8, 12.6, and 27.9 copies/mL for HIV-1 group M, HIV-1 group O, and HIV-2, respectively, with similar results for serum samples. LOD in dried blood spots was 255 copies/mL for HIV-1 and 984 copies/mL for HIV-2. HIV infection was detected 18.9 days and 8.5 days earlier than the confirmatory and serological assays, respectively, and at a similar time to the NAT.

Conclusions: The cobas HIV-1/2 Qual test enables early and accurate diagnoses of HIV-1 and HIV-2 in adults and children across sample types. The assay could help avert transmission during acute HIV, simplify HIV diagnostic algorithms, and promote the survival of HIV-infected children.

Key Words: HIV-1, HIV-2, HIV diagnostics, nucleic acid amplification tests, antigen infant diagnosis, acute HIV infection, dried blood spot

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BACKGROUND

Worldwide, there are an estimated 36.7 million people living with HIV and an additional 1.8 million new infections occur annually.[1] Only approximately 70% of all people living with HIV know their HIV status,[1] well short of the target of 90% set by The Joint United Nations Programme on HIV/AIDS.[2] After infection with HIV, there is a 3- to 4-week window, called acute HIV, before a serological response is detectable.[3–5] Fourth-generation tests that detect p24 protein antigen can identify infected individuals earlier in the course of the disease, at around 2–3 weeks after infection.[6] By targeting HIV RNA or DNA, nucleic acid amplification tests (NATs) can further reduce this window to about 10 days.[3,6]

Between 30% and 70% of individuals with acute HIV infection seek health care for symptoms that occur shortly after HIV infection.[7,8] An early HIV diagnosis allows for rapid treatment of the acute infection, which limits the size and genetic diversity of the viral reservoir, protects cells from persistent infection, and may enhance posttreatment

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control.[9–12] Detecting recently acquired infections is increasingly viewed as a core component of preventing horizontal and vertical transmission of HIV.[3] Although empirical evidence is sparse, modeling data suggest that as many as half of HIV infections in adults are acquired from people with acute or early HIV.[3,13] Similarly, rates of HIV infection in infants are severalfold higher than those in pregnant women with acute HIV compared with those with an established infection,[14] and acute HIV in pregnant women may account for as much as one-quarter of HIV infections in children.[15]

Although HIV-1 is responsible for most HIV infections, the prevalence of HIV-2 remains considerable in West Africa, and the strain has been reported worldwide.[16,17] Differentiating between HIV-1 and HIV-2 is important, given the varying clinical courses of these infections, the intrinsic resistance of HIV-2 to several antiretroviral drugs, and the need for different tests to monitor viral loads.[16,18–20] The World Health Organization (WHO) recommends that tests should be performed to distinguish HIV viral type in settings where HIV-2 is present.[21] The US Centers for Disease Control and Prevention and the European guidelines on HIV testing go further, stipulating that it is necessary to differentiate between HIV-1 and HIV-2 in all HIV-positive patients.[6,22,23] Many assays are limited to the identification of one HIV type, and those specifically designed for dual identification have high levels of serological cross-reactivity. In an assessment by WHO, cross-reactivity ranged from 3% to 57% with different assays and usually results in HIV-2 being overdiagnosed.[24–26] Importantly, both WHO and the US Food and Drug Administration require that screening and confirmatory serological tests must include detection of antibodies to both HIV-1 and HIV-2.

In addition to the global burden of HIV in adults, each year, an estimated 180,000 infants and young children acquire HIV.[27] Diagnosing HIV in infants and young children is challenging because antibodies from an HIV-infected mother pass through the placenta and through breastfeeding, making serological testing unreliable in children younger than 18 months.[28,29] In this age group, WHO recommends virological testing, using NAT or similar assays, to diagnose HIV, with testing performed at birth and at 4–6 weeks, and to confirm a positive serological test between 9 and 18 months.[30] At present, however, only about half of all HIV-exposed infants are tested within the first 2 months of life.[31] This is concerning because early diagnosis and treatment in infants can reduce HIV-related mortality and disease progression by 75% and enhance long-term cognitive outcomes, among other benefits.[32,33] Many strategies for increasing levels of HIV testing in children are based on the use of dried blood spots (DBSs) collected from finger pricks or other samples.[30,34] DBSs facilitate the decentralization of specimen collection, whereas maintaining high throughput at centralized laboratories.[35] Aside from DBSs' role in HIV testing in children, it has a broad range of applications within the HIV field, including monitoring antiretroviral treatment, diagnosing acute HIV infection, and estimating incidence in surveillance studies.[35,36]

The cobas HIV-1/HIV-2 qualitative test (cobas HIV-1/2 Qual; Roche Molecular Systems, South Branchburg, New Jersey.) for use on the cobas 6800/8800 Systems is the first CE-marked polymerase chain reaction (PCR) assay for the qualitative detection and differentiation of HIV-1 and HIV-2. This study evaluates the analytical and clinical performance of the assay using adult plasma and serum samples and pediatric DBS specimens.

METHODS

Study Procedures and Description of Device

This multicenter evaluation was conducted in Germany (Berlin and Ingelheim) and at National Health Laboratory Services, Johannesburg, South Africa. The protocol received ethical approval from the Ethikkommission der Universitätsmedizin Charité, Berlin (EA1/177/17), and the University of the Witwatersrand Human Research Ethics Committee (M150160). All specimens were unlinked and anonymized, and the study results were not used for patient management.

The cobas HIV-1/2 Qual test combines automated nucleic acid extraction and purification, with real-time PCR and result reporting separately for HIV-1 and HIV-2. It targets the HIV-1 long-terminal repeat and gag regions and HIV-2 long-terminal repeat region. For DBS testing, the assay required 70 μL of whole blood; spots were removed from the specimen collection card using disposable tweezers and transferred to Greiner Cryo.s tube (Fig. 1). Then, 1150 μL of cobas specimen preextraction reagent was added to each tube, which was placed in an Eppendorf Thermomixer and incubated for 10 minutes at 56°C and 1000 rpm. After incubation, the tubes were decapped, loaded, and processed on the cobas 6800/8800 instrument, together with tubes containing plasma and serum samples (650 μL volume in each). Handling of instruments, specimens, controls, and reagents was performed according to procedures described in the cobas 6800/8800 User Guide (version 3.0, Software version 1.2).

Method Correlation and Confirmation of HIV Infection

Four assessments were performed to validate the technical performance of the cobas HIV-1/2 Qual test. Assessments in adults were performed using leftover plasma and serum specimens from HIV-infected patients. Pediatric assay performance was evaluated using remnant DBS samples. Only samples with valid results for both the test under evaluation and the comparator tests were included in each evaluation (differences between the number of tests performed and results reported were due to invalid runs or samples). Discrepant samples were tested with heminested PCR and post-PCR ultraperformance liquid chromatography. These tests assisted in determining whether an observed signal was a true-positive result or a nonspecific amplification event. In addition, Elecsys HIV combi PT fourth-generation test (Roche Diagnostics GmbH, Penzberg, Germany) was used to confirm HIV-1–negative results in case of discordance, and sequencing analysis was used to confirm HIV-2–negative results.

The cobas HIV-1/2 Qual test results of plasma and serum samples (n = 339) were compared with those of the recomLine HIV-1 & HIV-2 Immunoglobulin G (IgG)

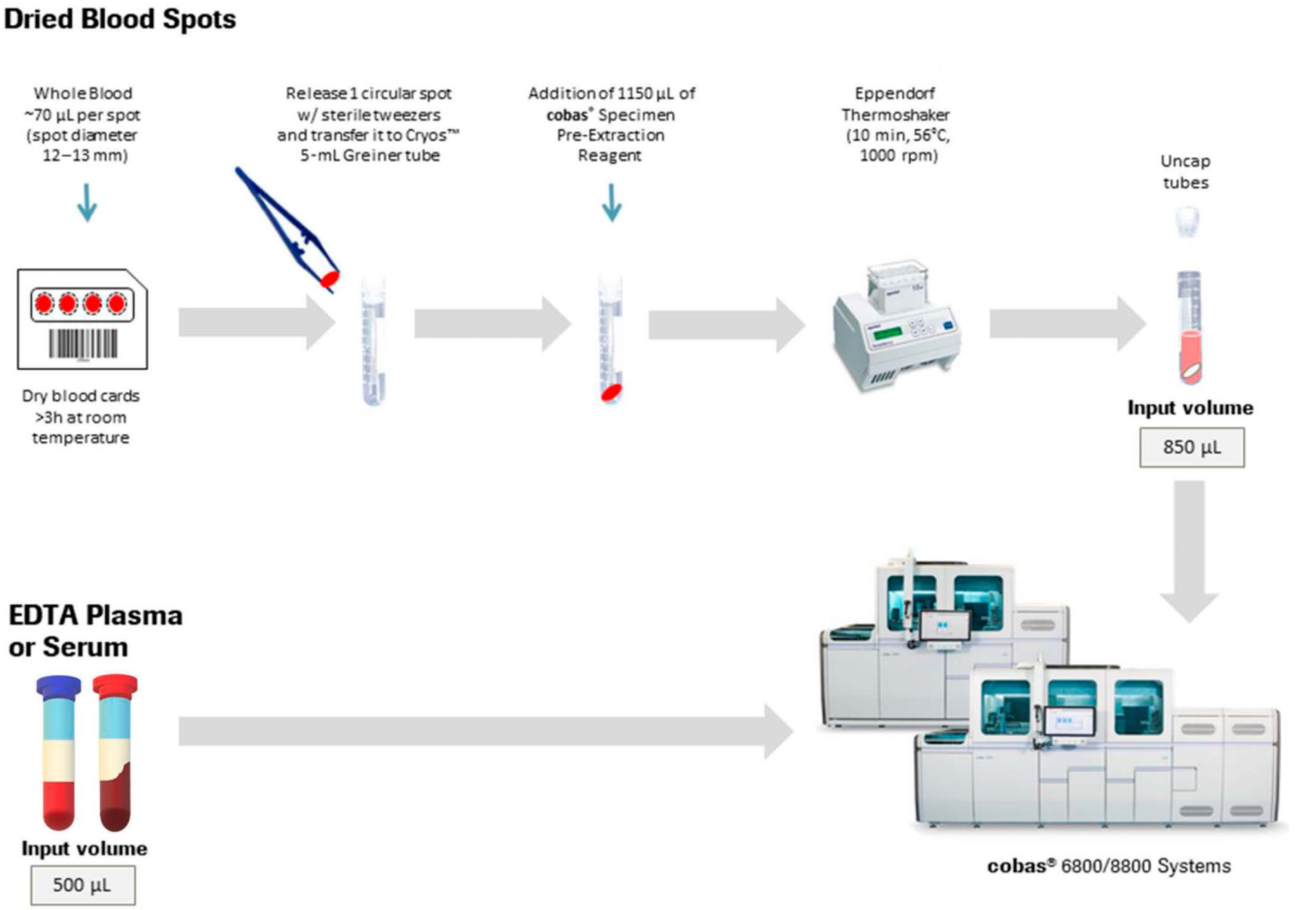

FIGURE 1. Overview of the cobas HIV-1/2 Qual test workflow for DBS.

(Mikrogen GmbH, Neuried, Germany), a CE-marked serological test that differentiates between HIV-1 and HIV-2. Plasma samples (n = 150) were compared with those on COBAS AmpliPrep/COBAS TaqMan (CAP/CTM), a CE-marked PCR test for HIV-1. Specimens were analyzed in single determinations. In addition, to assess the ability to detect HIV in patients who had not received antiretroviral treatment, 30 plasma and 30 serum samples from untreated patients with confirmed positive results for HIV-1 antigen and antibody were tested with the cobas HIV-1/2 Qual test.

Performance of cobas HIV-1/2 Qual test in early infant diagnosis was assessed against CAP/CTM using 311 DBSs from children aged 18 months or younger born to HIV-positive mothers. Samples were spotted onto Munktell TFN cards (n = 283) or Whatman 903 cards (n = 28).

Specificity

Specificity of cobas HIV-1/2 Qual test was determined by testing HIV-1/-2–negative plasma (n = 613), serum (n = 607), and DBS (n = 604) samples. Samples were collected from HIV-negative volunteers.

Genotype Inclusivity

To confirm genotype inclusivity of the cobas HIV-1/-2 Qual test, reference panels representing different HIV-1 and HIV-2 subtypes were analyzed. These panels consisted of HIV-1 group M subtypes A, C, D, F, G, H, J, and K and the circulating recombinant forms CRF01_AE, CRF02_AG, CRF12_BF, and CRF14_BG. Samples from HIV-1 groups N and O and HIV-2 groups A and B were also included. All specimens were previously confirmed to be HIV-positive with licensed serological tests and/or NATs and had HIV viral load levels commonly seen in infected patients. The reactivity of each target was determined in undiluted samples and in samples diluted in HIV-negative pooled plasma or serum to near the limit of detection (LOD) of the assay. For most genotypes, 10 panels were tested, but, because of limited availability, fewer panels were tested for HIV-1 group M subtypes J (5) and K (9), circulating recombinant forms CRF12_BF (2) and CRF14_BG (9), and HIV-1 group N (1).

Limits of Detection

Three independent dilution series were prepared consisting of 6 concentration levels for HIV-1 groups M and O and 5 concentration levels for HIV-2 (Table 4). The individual

intermediate stock solution aliquots were diluted in HIV-negative pooled plasma and serum. Dilution series were also prepared for DBSs in whole blood of 3 independent clinical samples.

Each panel was tested over multiple days, operators, systems, reagent lots, runs, and replicates per run. In total, with plasma and serum samples, 63 replicates per concentration level were tested for HIV-1 groups M and O, 42 replicates per concentration level were tested for HIV-2, and 84 DBS replicates per concentration level were tested for HIV-1 group M and HIV-2. For each target, the LOD was based on the probit value at the 95% hit rate, using the combined data from all lots. In addition, we determined the lowest concentration level with a ≥95% hit rate and the percentage of detection at 50% LOD using probit analysis.

Performance on Seroconversion Panels

We evaluated 35 HIV-1 group M commercially available seroconversion panels obtained from Zeptometrix, Inc. (Buffalo, NY) and Boston Biomedica, Inc-SeraCare Diagnostics (West Bridgewater, MA), each with a certificate of analysis. A single replicate was tested undiluted using the cobas HIV-1/2 Qual test. Three assessments were performed. The results of cobas HIV-1/2 Qual test were compared with the findings of a qualitative confirmatory assay for detecting antibodies to HIV-1 and HIV-2 (Bio-Rad Geenius HIV 1/2 Confirmatory Assay; Bio-Rad, Marnes-la-Coquette, France), a fourth-generation HIV immunoassay (Abbott ARCHITECT HIV Ag/Ab Combo test; Abbott, Wiesbaden, Germany), and a NAT (cobas TaqScreen MPX, v2.0). We reported the mean days to the first positive results and the difference in number of days to the detection between the cobas HIV-1/2 Qual test and the other assays.

Results

Method Correlation and Confirmation of HIV Infection

Concordance for HIV-infected samples between cobas HIV-1/2 Qual test and recomLine HIV-1 & HIV-2 IgG with plasma and serum samples was 100% for HIV-1 (302/302; 1 indeterminate test) and 99.7% for HIV-2 (301/302; Table 1). One result was indeterminate on recomLine HIV-1 & HIV-2 IgG and remained indeterminate on retesting. This sample showed negative result when retested in duplicate with Elecsys HIV combi PT fourth-generation test. Another sample was HIV-2–negative on cobas HIV-1/2 Qual test but HIV-2–positive on the recomLine HIV-1 & HIV-2 IgG. No HIV-2 sequence was obtained in sequencing analysis.

Comparison of cobas HIV-1/2 Qual test with the CAP/CTM showed 100% agreement for plasma samples (148/148; 68 HIV-1–positive and 80 HIV-1–negative). All 60 confirmed HIV-positive samples showed positive results with cobas HIV-1/2 Qual test, giving a sensitivity of 100%.

DBS samples from perinatally HIV-exposed children showed 99.6% agreement in cobas HIV-1/2 Qual test when compared with CAP/CTM (278/279). One sample showed a negative result for CAP/CTM, but positive results for cobas HIV-1/2 Qual test and for both heminested PCR and Post-PCR ultraperformance liquid chromatography. The overall sensitivity of the cobas HIV-1/2 Qual test was 100% (279/279) and specificity 99.3% (151/152).

TABLE 1. Cobas HIV-1/2 Qual Test Results Compared With recomLine HIV-1 & HIV-2 IgG Serological Test, CAP/CTM, and Predetermined HIV-Positive Specimens

Sample Category: Confirmed Results	Cobas HIV-1/2 Qual Test Result	Comparator Test Result		
		Positive	Negative	Indeterminate
The recomLine HIV-1 and HIV-2 IgG test result of plasma and serum				
HIV-1–positive EDTA plasma and serum (n = 138)§	Positive	138	0	0
HIV-1–negative EDTA plasma and serum (n = 165)§	Negative	0	164	1†
HIV-2–positive EDTA plasma and serum (n=14)§	Positive	14	0	0
HIV-2–negative EDTA plasma and serum (n = 289)§	Negative	1*	287	1†
CAP/CTM result for EDTA plasma and DBS specimens				
HIV-1–positive EDTA plasma (n = 68)§	Positive	68	0	0
HIV-1–negative EDTA plasma (n = 80)§	Negative	0	80	0
Pediatric HIV-1–positive DBSs (n = 128)§	Positive	127	1‡	0
Pediatric HIV-1–negative DBSs (n = 151)§	Negative	0	151	0
Predetermined HIV antibody/antigen-positive samples				
HIV-Positive EDTA plasma and serum (60)	Positive	60	0	0

*No HIV-2 sequence detected in sequencing analysis.
†HIV-1–negative on Elecsys HIV combi PT test.
‡HIV-1–positive on heminested PCR and post-PCR ultraperformance liquid chromatography.
§McNemar exact test, $P = 1.0$. Specimens from adults unless indicated.

Specificity

Four DBS samples showed positive results for HIV-1 on cobas HIV-1/2 Qual test. These were excluded after testing on heminested PCR, which confirmed that the samples were true HIV-1–positive specimens. All valid plasma (n = 613), serum

TABLE 2. Specificity of Cobas HIV-1/2 Qual Test for HIV-Negative Plasma, Serum, and Dried Blood Spot Specimens

	HIV-1 Results, n (%)		HIV-2 Results, n (%)		
Sample Type	**Negative**	**Positive**	**Negative**	**Positive**	**Specificity (%), (95% CI)**
EDTA plasma (n = 613)	613 (100)	0 (0)	613 (100)	0 (0)	100.0 (99.5 to 100)
Serum (n = 607)	607 (100)	0 (0)	607 (100)	0 (0)	100.0 (99.5 to 100)
DBSs (n = 604)	604 (100)	0 (0)	604 (100)	0 (0)	100.0 (99.5 to 100)

(n = 607), and DBS samples (n = 604) tested HIV-negative with cobas HIV-1/2 Qual test. The specificity of cobas HIV-1/2 Qual test on each sample matrix was, thus, 100%, with a lower one-sided 95% confidence interval of 99.5% (Table 2).

Genotype Inclusivity

We observed test positivity rates of 100% for all HIV-1 and HIV-2 groups and subtypes tested in undiluted samples (Table 3). Similarly, 100% subtype inclusivity was demonstrated in dilutions with all subtypes at about 5xLOD, aside from HIV-1 group N. The one HIV-1 group N cultured isolate was detected in 4 replicates at several dilutions, including at about 3 × LOD, but was detected in only 50% of cases at a dilution substantially below the LOD.

Limits of Detection

The LOD determined using probit analysis for plasma LODs was 13 copies/mL for HIV-1 group M, 15 copies/mL for HIV-1 group O, and 28 copies/mL for HIV-2 (Table 4). The corresponding LODs for serum were 12, 13, and 23 copies/mL. The LOD in DBS for HIV-1 group M was 255 copies/mL and 984 copies/mL for HIV-2. The LOD determined by ≥95% hit rate on plasma and serum was 20 copies/mL for HIV-1 groups M and O and 360 copies/mL for DBS HIV-1 group M dilutions (for HIV-2 ≥95% hit rates, refer to Table 4). The 50% LOD estimation using probit analysis for plasma LODs was 2.9 copies/mL for HIV-1 group M, 3.4 copies/mL for HIV-1 group O, and 5.8 copies/mL for HIV-2 (Table 4). The corresponding 50% LODs for serum were 3.0, 2.8, and 6.1 copies/mL. The LOD in DBS for HIV-1 group M was 57.1 copies/mL and 227.3 copies/mL for HIV-2.

Performance on Seroconversion Panels

In the first assessment, consisting of 10 panels, a difference in days to first test positivity could not be determined in 4 cases because the Bio-Rad Geenius HIV 1/2 Confirmatory Assay remained negative at the last visit day (Fig. 2). One panel member was excluded because its first day of detection varied considerably from the other panels, both on the certificate of analysis (day 97) and on HIV-1/2 Qual (day 127). In the remaining 5 panels, cobas HIV-1/2 Qual test detected HIV-1 a mean of 18.9 days earlier than the Bio-Rad Geenius HIV 1/2 Confirmatory Assay (Fig. 2). The second assessment, testing of 25 panels, found that cobas HIV-1/2 Qual test detected HIV-1 a mean of 8.5 days earlier than the Abbott ARCHITECT HIV Ag/Ab Combo assay (Fig. 3). Finally, in 20 of the 25 panels tested in the third assessment, the number of days to reactive result was identical in the cobas HIV-1/2 Qual test and cobas TaqScreen MPX, v2.0. The cobas HIV-1/2 Qual test detected HIV-1 earlier in 2 of the 25 panels by an average of 8 days and later in 3 panels by a mean of 3.3 days.

TABLE 3. Inclusivity Panels

HIV Group	HIV Subtype	Samples Undiluted, n reactive/N tested (% Reactive Rate)	Samples Diluted (~5X LOD), n reactive/N tested (% Reactive Rate)
HIV-1 group M	A	10/10 (100)	10/10 (100)
	C	10/10 (100)	10/10 (100)
	D	10/10 (100)	10/10 (100)
	F	10/10 (100)	10/10 (100)
	G	10/10 (100)	10/10 (100)
	H	10/10 (100)	10/10 (100)
	J	5/5 (100)	5/5 (100)
	K	9/9 (100)	9/9 (100)
	CRF01_AE	10/10 (100)	10/10 (100)
	CRF02_AG	10/10 (100)	10/10 (100)
	CRF12_BF	2/2 (100)	2/2 (100)
	CRF14_BG	9/9 (100)	9/9 (100)
HIV-1 group N	—	1/1 (100)	1/1 (100)
HIV-1 group O	—	10/10 (100)	10/10 (100)
HIV-2 group A	—	10/10 (100)	10/10 (100)
HIV-2 group B	—	10/10 (100)	10/10 (100)

TABLE 4. Limits of Detection for EDTA Plasma, Serum, and Dried Blood Spots

Sample Type	Target	Concentration Levels	Standards	Panels (n Dilution Series)	cobas 6800/8800 Systems (n)	Replicates (n)	Total Valid Replicates (n)	50% Probit LOD cp/mL (95% CI)	95% Probit LOD cp/mL (95% CI)	LOD by ≥95% Hit Rate (cp/mL)
EDTA plasma	HIV-1 group M	6	Third HIV-1 WHO International Standard, HIV-1 group M, subtype B	3	5	63	1134	2.9 (2.1 to 3.6)	13 (10.9 to 15.2)	20
	HIV-1 group O	6	HIV-1 Group O Roche Primary Standard	3	5	63	1134	3.4 (3.0 to 3.8)	15 (12.8 to 17.7)	20
	HIV-2 group A*	5	HIV-2 first International WHO Standard	3	4	42	630	5.8 (4.8 to 6.7)	27.9 (22.9 to 36.6)	40
Serum	HIV-1 group M	6	Third HIV-1 WHO International Standard, HIV-1 group M, subtype B	3	5	63	1134	3.0 (2.6 to 3.3)	12 (10.5 to 14.5)	20
	HIV-1 group O	6	HIV-1 Group O Roche Primary Standard	3	5	63	1134	2.8 (2.4 to 3.2)	13 (10.9 to 15.2)	20
	HIV-2 group A*	5	HIV-2 first International WHO Standard	3	4	42	630	6.1 (5.2 to 6.9)	23 (19.6 to 29.7)	40
DBSs†	HIV-1 group M	6	Third HIV-1 WHO International Standard, HIV-1 group M, subtype B	3	4	84	1505	57.1 (51.1 to 63)	255 (223.7 to 299.1)	360
	HIV-2 group A	5	HIV-2 Roche Primary Standard	3	4	84	1243	227.3 (203.1 to 250.5)	984 (856.2 to 1169.0)	1450

*For plasma and serum, the 50% and 95% probit LODs were calculated with a minimum of 180 replicates per concentration level, except where indicated by (*), where a minimum of 120 replicates were used.

†For DBS, the 50% and 95% probit LODs calculated with minimum of 246 valid replicates per concentration level (†).

cp/mL, copies per milliliter.

DISCUSSION

The cobas HIV-1/2 Qual test demonstrated excellent sensitivity, specificity, and genotype inclusivity for both HIV-1 and HIV-2 in plasma, serum, and DBS samples. The assay also detected HIV several weeks earlier than an HIV antibody test and a fourth-generation antibody/antigen test. Furthermore, correlation of the cobas HIV-1/2 Qual test with the recomLine HIV-1 & HIV-2 IgG and CAP/CTM showed more

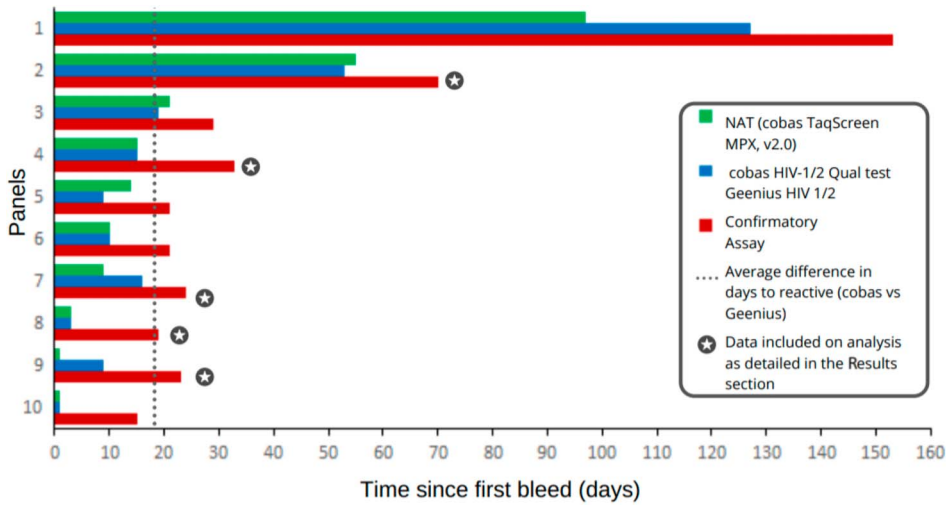

FIGURE 2. HIV-1 seroconversion panel results comparing time to diagnosis with the cobas HIV-1/2 Qual test and Bio-Rad Geenius HIV 1/2 Confirmatory Assay.

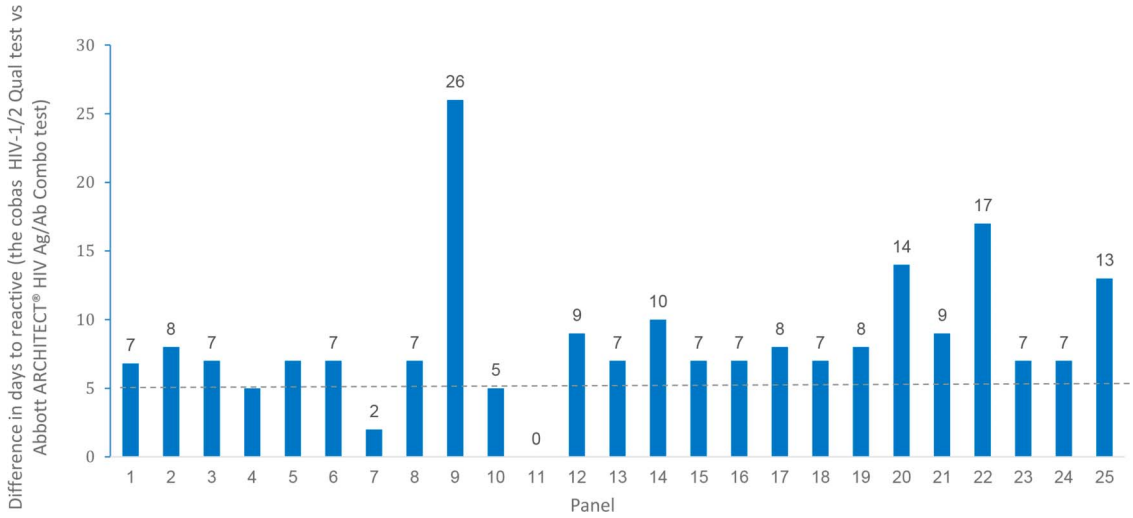

FIGURE 3. HIV-1 seroconversion panel results comparing time to diagnosis with the cobas HIV-1/2 Qual test and Abbott ARCHITECT HIV Ag/Ab Combo test.—Average difference in days to reactive (cobas vs ARCHITECT).

than 99% concordance across all sample types. No cross-reactivity with HIV-1 and HIV-2 was noted. In a few discordant samples, the results of cobas HIV-1/2 Qual test concurred with those of additional tests performed to resolve these discrepancies. We observed reactivity rates of 100% for all HIV-1 and HIV-2 genotypes in undiluted specimens from HIV-positive patients and in all dilutions above the LOD. The performance of the HIV cobas HIV-1/2 Qual test documented in this study suggests that the test is suitable for the second test on the CDC and WHO testing algorithm. Negative RNA samples can then be reflexed to the Geenius assay for final confirmation. Such decisions are based on cost, test availability, and laboratory capacity, among other factors.

Among adults, the ability of HIV cobas HIV-1/2 Qual test to detect HIV shortly after infection has considerable implications, both for the index patient and for their sexual or injecting drug partners.[3,4] Furthermore, oral preexposure prophylaxis to prevent HIV acquisition, and potentially injectable preexposure prophylaxis in future, presents major diagnostics concerns, especially the lengthy delays in seroconversion that may occur. Early diagnosis is important in people taking preexposure prophylaxis and before commencing prophylaxis because they may develop resistance to the antiretroviral drug if diagnosis is delayed. Tests in this context involving plasma, DBS, or even lysed whole blood need to have low LODs because the viral load levels may be low in these patients, given they are taking antiretroviral drugs. Moreover, timely diagnosis of HIV can reduce the risk of HIV transmission to infants by detecting new HIV infections in pregnant and breastfeeding women and can raise survival and minimize HIV-related morbidity in children who do acquire HIV.[33] Early diagnosis of HIV is also a central part of the care packages for individuals requiring HIV postexposure prophylaxis after sexual, occupational, or other exposures to HIV.[30] In addition, the assay could play an important role in detecting resistant virus and poor adherence in people receiving antiretroviral treatment.

The ability of the test to discriminate between HIV-1 and HIV-2 means that the cobas HIV-1/2 Qual assay has the potential to decrease the number of NATs needed in diagnostic algorithms that include HIV-1 and HIV-2. In addition, this feature of the assay may allow type discrimination to be extended beyond the countries where this is currently recommended. This would have major implications for people infected with HIV-2 who are currently undiagnosed in most settings and, as a consequence, receive suboptimal care.[20]

The study highlights several important evidence gaps in this field. A major concern relates to the accuracy of early infant diagnosis assays.[37] As rates of mother-to-child transmission of HIV decline, so does the positive predictive value of these assays. As many as 10% of infants who initiate treatment in settings with highly effective programs for preventing mother-to-child transmission may have false-positive diagnoses.[38] In 2018, WHO, thus, recommended the use of an "indeterminate range" in NAT tests as a means of optimizing the trade-off between the harms of incorrectly classifying an HIV-infected infant as indeterminate and the harms of starting treatment in HIV-uninfected infants.[37] Conversely, false-negative results are also highly worrisome among newborns, infants, and young children because antiretroviral drugs taken by the mother during pregnancy or breastfeeding or by the child may cause low-level viremia in infected children.[30,39,40] More broadly, the results of this study will need to be confirmed in field conditions.

In conclusion, the cobas HIV-1/2 Qual test is a CE-marked real-time PCR assay that identified acute HIV infection earlier than the fourth-generation tests and reliably differentiated between HIV-1 and HIV-2. The assay performed well on a range of sample types and in both adults and children. The test could be considered for inclusion in HIV testing guidelines in the United States and the European Union and in other settings where differentiation of HIV-1 and HIV-2 is currently recommended. Of note, the assay

could simplify HIV diagnostic algorithms and expand access to NAT HIV testing for adults and children through the use of DBS samples. These features of the test mean that it could make a substantial contribution to reaching the "first 90" The Joint United Nations Programme on HIV/AIDS goal, the testing of 90% of the population.[2] Finally, earlier detection of HIV has important survival benefits for children and reduces HIV transmission among adults.

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