Early diagnosis of HIV-1 and HIV-2 using cobas HIV-1/HIV-2 Qualitative, a novel qualitative nucleic acid amplification test for plasma, serum and dried blood spot specimens

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ABSTRACT

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

**Methods:** We evaluated the performance of the cobas HIV-1/HIV-2 Qualitative Test (“cobas HIV-1/2 Qual”) for use on the cobas 6800/8800 Systems. Results on adult plasma and serum samples, and paediatric dried blood spots were compared with recomLine HIV-1 & HIV-2 IgG serological test and COBAS AmpliPrep/COBAS TaqMan HIV-1 Qualitative Test, v2.0 (“CAP/CTM”). Genotype inclusivity and limits of detection (LOD) were determined, and sensitivity on seroconversion panels compared to Bio-Rad Geenius HIV 1/2 Confirmatory Assay, Abbott ARCHITECT HIV Ag/Ab Combo serologic test and cobas TaqScreen MPX, v2.0.

**Results:** Concordance of cobas HIV-1/2 Qual with the comparator serologic test and CAP/CTM was ≥99.6% with all sample types. Reactivity with all HIV genotypes was 100%. LOD in plasma samples was 14.8 copies/mL for HIV-1 group M, 12.6 copies/mL for HIV-1 group O and 27.9 copies/mL for HIV-2, with similar results for serum. Dried blood spots LOD 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 serologic assays, respectively; and at a similar time to the NAT.

**Conclusions:** cobas HIV-1/2 Qual test enables early and accurate diagnosis 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.

**Keywords:** HIV-1; HIV-2; HIV diagnostics; Nucleic acid amplification tests; antigen infant diagnosis; acute HIV infection; dried blood spot

**Background**

Worldwide, there are an estimated 36.7 million people living with HIV and an additional 1.8 million new infections occur annually. Only about 70% of all people living with HIV know their HIV status, well short of the target of 90% set by UNAIDS. Following infection with HIV, there is a three- to four-week window, called acute HIV, before a serologic response is detectable. Fourth-generation tests that detect p24 protein antigen can identify infected individuals earlier in the course of the disease, at around two to three weeks after infection. By targeting HIV RNA or DNA, nucleic acid amplification tests (NATs), can further reduce this window to around 10 days.

Between 30 to 70% of individuals with acute HIV infection seek healthcare for symptoms that occur shortly after HIV infection. 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 post-treatment control. Detecting recently acquired infections is increasingly viewed as a core component of preventing horizontal and vertical transmission of HIV. Though empirical evidence is sparse, modelling data suggests that
as many as half of HIV infections in adults are acquired from people with acute or early HIV\textsuperscript{3,13}. Similarly, rates of HIV infection in infants are several-fold higher in pregnant women with acute HIV compared to those with established infection\textsuperscript{14}, and acute HIV in pregnant women may account for as much as one quarter of HIV infections in children\textsuperscript{15}.

Though HIV-1 is responsible for the vast majority of HIV infections, the prevalence of HIV-2 remains considerable in West Africa and the strain has been reported worldwide\textsuperscript{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\textsuperscript{16,18-20}. WHO recommends that tests should be performed to distinguish HIV viral type in settings where HIV-2 is present\textsuperscript{21}. The United States 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\textsuperscript{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 over-diagnosed\textsuperscript{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\textsuperscript{27}. Diagnosing HIV in infants and young children is challenging as antibodies from an HIV-infected mother pass through the placenta and via breastfeeding, making serological testing unreliable in children under 18 months\textsuperscript{28,29}. In this age group, WHO recommends virological testing, using NAT or similar assays, to diagnose HIV, with testing done
at birth and at 4-6 weeks, as well as to confirm a positive serological test between 9 and 18 months \(^{30}\). Presently, however, only about half of all HIV-exposed infants are tested within the first two months of life \(^{31}\). This is concerning since 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, while 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 for 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, Inc.) 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 analytical and clinical performance of the assay, using adult plasma and serum samples, as well as paediatric DBS specimens.

**Methods**

**Study procedures and description of device**

This was a multi-centre evaluation 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, anonymised 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 requires 70 µL of whole blood, spots are removed from the specimen collection card using disposable tweezers and transferred to Greiner Cryo.s tube (Figure 1). Then, 1150 µL of cobas Specimen Pre-Extraction Reagent is added to each tube, which is placed in an Eppendorf Thermomixer and incubated for 10 minutes at 56°C and 1000rpm. Following incubation, the tubes are de-capped, 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 are carried out 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 done using left-over plasma and serum specimens from HIV-infected patients. Paediatric assay performance was evaluated using remnant DBS samples. Only samples with valid results on both the test under evaluation and the comparator tests were included in each evaluation (differences between number of tests done and results reported are due to invalid runs or samples). Discrepant samples were tested with Hemi-Nested PCR and Post PCR Ultra Performance Liquid Chromatography (UPLC). These tests assisted in determining if an observed signal was a true positive result or a non-specific amplification event. Additionally, Elecsys HIV combi PT fourth generation test (Roche Diagnostics. GmbH, Penzberg, Germany) was used to confirm negative HIV-1 results in case of discordance and sequencing analysis used for confirming HIV-2 negative results.
The cobas HIV-1/2 Qual test results on plasma and serum samples (n=339) were compared to the recomLine HIV-1 & HIV-2 IgG (Mikrogen GmbH, Neuried, Germany), a CE-marked serological test that differentiates between HIV-1 and HIV-2. Plasma samples (n=150) were compared to CAP/CTM, a CE-marked PCR test for HIV-1. Specimens were analysed in single determinations. In addition, to assess ability to detect HIV in patients who had not received antiretroviral treatment, 30 plasma and 30 serum samples from untreated patients who were confirmed antigen and antibody positive for HIV-1 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 DBS from children ≤18 months 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/HIV-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 analysed. These panels consisted of HIV-1 group M subtypes A, C, D, F, G, H, J, 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 serologic 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, ten panels were tested, but, due to limited availability, fewer panels were tested for HIV-1 group M subtypes J (5), K (9), CRF12_BF (2) and CRF14_BG (9), and HIV-1 group N (1).

**Limits of detection**

Three independent dilution series were prepared consisting of six concentration levels for HIV-1 group M and HIV-1 group O, and five 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 three 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 of 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 BBI-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 done. Results on cobas HIV-1/2 Qual were compared with
findings of a qualitative confirmatory assay for detecting antibodies to HIV-1 and HIV-2 (Bio-
Rad Geenius HIV 1/2 Confirmatory Assay), a fourth-generation HIV immunoassay (Abbott
ARCHITECT HIV Ag/Ab Combo test) and a NAT (cobas TaqScreen MPX, v2.0). We report the
mean days to first positive results as well as the difference in number of days to detection
between the cobas HIV-1/2 Qual and the other assays.

Results

Method correlation and confirmation of HIV infection

Concordance for HIV-infected samples between cobas HIV-1/2 Qual 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 was negative when retested in
duplicate with Elecsys HIV combi PT fourth generation test. Another sample was HIV-2
negative on cobas HIV-1/2 Qual, 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 with the CAP/CTM showed 100% agreement for plasma
samples (148/148; 68 HIV-1 positive, 80 HIV-1 negative). All 60 samples on the confirmed
HIV-positive samples were positive with cobas HIV-1/2 Qual test, giving a sensitivity of 100%.

DBS samples from perinatally HIV-exposed children showed 99.6% agreement on cobas HIV-
1/2 Qual when compared with CAP/CTM (278/279). One sample was negative on CAP/CTM,
but positive on cobas HIV-1/2 Qual, and on both Hemi-Nested PCR and post PCR UPLC. The
overall sensitivity of the cobas HIV-1/2 Qual test was 100% (279/279) and specificity 99.3%
(151/152).
Specificity

Four DBS samples tested positive for HIV-1 on cobas HIV-1/2 Qual. These were excluded after testing on Hemi-Nested PCR, which confirmed that the samples were true HIV-1 positive specimens. All valid plasma (n=613), serum (n=607) and DBS samples (n=604) tested HIV negative with cobas HIV-1/2 Qual. The specificity of cobas HIV-1/2 Qual 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 around 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 around 3xLOD, 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 copies/mL, 13 copies/mL 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 group 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).
corresponding 50% LODs for serum were 3.0 copies/mL, 2.8 copies/mL 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 as the Bio-Rad Geenius HIV 1/2 Confirmatory Assay remained negative at the last visit day (Figure 2). One panel member was excluded as 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 detected HIV-1 a mean 18.9 days earlier than the Bio-Rad Geenius HIV 1/2 Confirmatory Assay (Figure 2). The second assessment, testing of 25 panels, found that cobas HIV-1/2 Qual detected HIV-1 a mean 8.5 days earlier than the Abbott ARCHITECT HIV Ag/Ab Combo assay (Figure 3). Lastly, in 20 of 25 panels tested in the third assessment, the number of days to reactive result was identical in the cobas HIV-1/2 Qual and cobas TaqScreen MPX, v2.0. cobas HIV-1/2 Qual detected HIV-1 earlier in 2 of 25 panels by an average of 8 days, and later in 3 panels by a mean 3.3 days.

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. Further, correlation of the cobas HIV-1/2 Qual with the recomLine HIV-1 & HIV-2 IgG and CAP/CTM showed over 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 concurred with those of additional tests done 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 documented in this study suggests the test is suitable for the second test on the CDC and WHO testing algorithm. Negative RNA samples can then be reflexed to the Ge嫩ius 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 to detect HIV shortly after infection has considerable implications, both for the index patient and their sexual or injecting drug partners. Further, oral pre-exposure prophylaxis to prevent HIV acquisition, and potentially injectable pre-exposure prophylaxis in future, present major diagnostics concerns, especially the lengthy delays in seroconversion which may occur. Early diagnosis is important in people taking pre-exposure prophylaxis and prior to commencing prophylaxis, as 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 as 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. Early diagnosis of HIV is also a central part of the care packages for individuals requiring HIV post-exposure prophylaxis after sexual, occupational or other exposures to HIV. Additionally, the assay could play an important role in detecting resistant virus and poor adherence in people receiving antiretroviral treatment.

The test’s ability 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. Additionally, 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 sub-optimal 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 programmes for preventing mother-to-child transmission may have false-positive diagnoses.³⁸ In 2018, WHO thus recommended use of an ‘indeterminate range’ in NAT tests as a means of optimising 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 as 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 is a CE-marked real-time PCR assay that identifies acute HIV infection earlier than fourth generation tests and reliably differentiates between HIV-1 and HIV-2. The assay performs 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 use of DBS samples. These features of the test mean it could make a substantial contribution to reaching the ‘first 90’ UNAIDS goal, the
testing of 90% of the population\(^2\). Lastly, earlier detection of HIV has important survival benefits for children and reduces HIV transmission among adults.

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**References**


Figure legends:

**Figure 1:** Overview of the cobas HIV-1/2 Qual test workflow for dried blood spots

**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)
Table 1: cobas HIV-1/2 Qual test results compared to recomLine HIV-1 & HIV-2 IgG serological test, CAP/CTM and predetermined HIV-positive specimens

<table>
<thead>
<tr>
<th>Sample category: Confirmed results</th>
<th>cobas HIV-1/2 Qual test result</th>
<th>Comparator test result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
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<tr>
<td>recomLine HIV-1 and HIV-2 IgG test result on plasma and serum</td>
<td>Positive</td>
<td>138</td>
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<td>HIV-1 positive EDTA plasma and serum (n=138)*</td>
<td>Positive</td>
<td>HIV-1 negative EDTA plasma and serum (n=165)*</td>
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<td>HIV-2 positive EDTA plasma and serum (n=14)*</td>
<td>Positive</td>
<td>14</td>
</tr>
<tr>
<td>HIV-2 negative EDTA plasma and serum (n=289)*</td>
<td>Negative</td>
<td>1a</td>
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<tr>
<td>CAP/CTM result for EDTA plasma and dried blood spot specimens</td>
<td>Positive</td>
<td>68</td>
</tr>
<tr>
<td>HIV-1 positive EDTA plasma (n=68)*</td>
<td>Positive</td>
<td>0</td>
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<tr>
<td>HIV-1 negative EDTA plasma (n=80)*</td>
<td>Negative</td>
<td>Paediatric HIV-1 positive dried blood spots (n=128)*</td>
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<td>Paediatric HIV-1 negative dried blood spots (n=151)*</td>
<td>Negative</td>
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</tr>
<tr>
<td>Predetermined HIV antibody/antigen positive samples</td>
<td>Positive</td>
<td>60</td>
</tr>
</tbody>
</table>

EDTA ethylenediaminetetraacetic acid. *No HIV-2 sequence detected in sequencing analysis; 1a HIV-1 negative on Elecsys HIV combi PT test; 1b HIV-1 positive on Hemi Nested PCR and Post PCR Ultra Performance Liquid Chromatography. 1c McNemar Exact Test P=1.0. Specimens from adults unless indicated
Table 2 Specificity of cobas HIV-1/2 Qual test for HIV-negative plasma, serum and dried blood spot specimens

<table>
<thead>
<tr>
<th>Sample type</th>
<th>HIV-1 results</th>
<th>HIV-2 results</th>
<th>Specificity (%) (95% CI)</th>
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<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
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<td>EDTA plasma (n=613)</td>
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<tr>
<td>Serum (n=607)</td>
<td>607 (100)</td>
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<td>607 (100)</td>
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<tr>
<td>Dried blood spots (n=604)</td>
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<td>604 (100)</td>
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</tbody>
</table>

CI confidence interval. EDTA ethylenediaminetetraacetic acid.
Table 3 Inclusivity panels

<table>
<thead>
<tr>
<th>HIV group</th>
<th>HIV subtype</th>
<th>Samples undiluted n reactive/N tested (% reactive rate)</th>
<th>Samples diluted (~5X LOD) n reactive/N tested (% reactive rate)</th>
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<tbody>
<tr>
<td>HIV-1 group M</td>
<td>A</td>
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<td>HIV-1 group M</td>
<td>C</td>
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<td>HIV-1 group O</td>
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<td>10/10 (100)</td>
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</tr>
<tr>
<td>HIV-2 group B</td>
<td>-</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
</tr>
</tbody>
</table>

LOD, limit of detection
Table 4: Limits of detection for EDTA plasma, serum and dried blood spots

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Target</th>
<th>Concentration levels</th>
<th>Standards</th>
<th>Panels (n dilution series)</th>
<th>cobas® 6800/8800 systems (n)</th>
<th>Replicates (n)</th>
<th>Total valid replicates (n)</th>
<th>50% probit LOD cp/mL (95% CI)</th>
<th>95% probit LOD cp/mL (95% CI)</th>
<th>LOD by ≥95% hit rate (cp/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA plasma</td>
<td>HIV-1 group M</td>
<td>6</td>
<td>3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B</td>
<td>3</td>
<td>5</td>
<td>63</td>
<td>1134</td>
<td>2.9 (2.1-3.6)</td>
<td>13 (10.9-15.2)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HIV-1 group O</td>
<td>6</td>
<td>HIV-1 Group O Roche Primary Standard</td>
<td>3</td>
<td>5</td>
<td>63</td>
<td>1134</td>
<td>3.4 (3.0-3.8)</td>
<td>15 (12.8-17.7)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HIV-2 group A*</td>
<td>5</td>
<td>HIV-2 1st International WHO Standard</td>
<td>3</td>
<td>4</td>
<td>42</td>
<td>630</td>
<td>5.8 (4.8-6.7)</td>
<td>27.9 (22.9-36.6)</td>
<td>40</td>
</tr>
<tr>
<td>Serum</td>
<td>HIV-1 group M</td>
<td>6</td>
<td>3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B</td>
<td>3</td>
<td>5</td>
<td>63</td>
<td>1134</td>
<td>3.0 (2.6-3.3)</td>
<td>12 (10.5-14.5)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HIV-1 group O</td>
<td>6</td>
<td>HIV-1 Group O Roche Primary Standard</td>
<td>3</td>
<td>5</td>
<td>63</td>
<td>1134</td>
<td>2.8 (2.4-3.2)</td>
<td>13 (10.9-15.2)</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>HIV-2 group A*</td>
<td>5</td>
<td>HIV-2 1st International WHO Standard</td>
<td>3</td>
<td>4</td>
<td>42</td>
<td>630</td>
<td>6.1 (5.2-6.9)</td>
<td>23 (19.6-29.7)</td>
<td>40</td>
</tr>
<tr>
<td>Dried blood spots**</td>
<td>HIV-1 group M</td>
<td>6</td>
<td>3rd HIV-1 WHO International Standard, HIV-1 group M, subtype B</td>
<td>3</td>
<td>4</td>
<td>84</td>
<td>1505</td>
<td>57.1 (51.1, 63)</td>
<td>255 (223.7-299.1)</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td>HIV-2 group A*</td>
<td>5</td>
<td>HIV-2 Roche Primary Standard</td>
<td>3</td>
<td>4</td>
<td>84</td>
<td>1243</td>
<td>227.3 (203.1-250.5)</td>
<td>984 (856.2-1169.0)</td>
<td>1450</td>
</tr>
</tbody>
</table>

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 (**)
Figure 1: Overview of the cobas HIV-1/2 Qual test workflow for dried blood spots
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)