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SAMBA HIV semi-quantitative test, a new point-of-care viral load monitoring assay for resource-limited settings.

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Running Title: SAMBA HIV-1 Semi-Q evaluation

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Abstract

Routine viral load (VL) testing of HIV-infected individuals on antiretroviral therapy (ART) is used to monitor treatment efficacy. However, due to logistical challenges, implementation of VL has been difficult in resource-limited settings. The aim of this study was to evaluate the performance of the SAMBA Semi-Q Test in London, Malawi, and Uganda. The SAMBA HIV-1 Semi-Q Test can distinguish between patients with VL above or below 1000 copies/ml. The SAMBA Semi-Q was validated with diluted clinical samples and blinded plasma samples collected from HIV-1–positive individuals. SAMBA Semi-Q results were compared with results from the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2.0. Testing of 96 2-10 fold dilutions of four samples containing HIV-1 subtype C as well as 488 samples from patients in the United Kingdom, Malawi, and Uganda, respectively, yielded an overall accuracy for SAMBA Semi-Q of 99% (95% CI 93.8 – 99.9%) and 96.9% (95% CI 94.9 – 98.3%) respectively compared to Roche. Analysis of VL data from patients in Malawi and Uganda showed that the SAMBA cut-off of 1000 copies/ml appropriately distinguished treated from untreated individuals. Furthermore, analysis of the viral load of 232 patients on ART in Malawi and Uganda revealed similar patterns for virological control defined as either <1000 copies/ml (SAMBA cut-off) or <5000 copies/ml (WHO 2010 criterion). This study suggests that SAMBA Semi-Q has adequate concurrency with the gold standard measurements for viral load measurement. This test can allow VL monitoring of patients on ART at the point of care in resource-limited settings.

Introduction

There have been steady improvements in scaling-up access to antiretroviral therapy (ART) in resource-limited countries.(1) There appears to be fewer new infections and AIDS related deaths have decreased over the past decade. While these achievements are remarkable, there remains a large unmet need, given that 34 million people are living with HIV/AIDS globally; most of whom live in sub-Saharan Africa.

Effective ART not only improves the survival of individuals infected with HIV but also prevents transmission.(2) The global public health community is therefore committed to achieving universal access to HIV treatment, with a target of increasing the availability of ART to 15 million people by the end of 2015.(3) Effective ART suppresses HIV replication, which is measured through plasma viral load (VL), specifically looking at potential adherence or treatment failure. VL monitoring prolongs the duration on first-line regimens by preventing unnecessary switches in ART to more complex and expensive second-line regimens that result in increased costs and decrease in drug options (4, 5) and can reduce the delay in switching to second-line drugs or patient counseling, resulting in better patient outcomes.(6, 7) Studies have shown that sites with VL monitoring have lower mortality rates (6) and better justification of switching to 2nd line drug regimens than at sites using only CD4. (4)

Quantitative VL is the standard of care for patients receiving ART in resource-rich settings.(8, 9) In resource-poor settings, however, laboratory diagnostics are often only available at centralized laboratories in major cities due to the complexity of the technology, the infrastructure and trained personnel required for the tests.(10-12) As ART programs

have scaled up, there has been a significant effort to decentralize care to local primary health centers, which have basic services and limited infrastructure. To access VL testing, peripheral HIV-treatment facilities must transport patient specimens to central laboratories under optimal conditions within a limited period of time, followed by testing and return of results. This results in increased costs of service delivery and unacceptable delays in obtaining test results with consequent losses to follow-up.(13) A priority focus area of the Treatment 2.0 initiative is therefore the development of affordable, reliable, quality-assured, point-of-care molecular diagnostic platforms. (14)

SAMBA (Simple Amplification-Based Assay) HIV-1 Semi-quantitative Test (SAMBA HIV-1 Semi-Q) has been developed as a robust, simple, and relatively rapid point-of-care test to distinguish between patients with a VL above or below 1000 copies/ml within 90 minutes. The main advantages of SAMBA include visual detection of the result and robust reagents, which are stable at high temperatures and humidity. All reagents required are preloaded in single-use, disposable cartridges to ensure that the system is easy to use and to prevent potential contamination of the laboratory with amplified product. The chemistry is based on the SAMBA Qualitative Test and can therefore detect all known HIV-1 subtypes. (15) In consultation with experts in the field of ART provision in low and middle income countries (LMIC), Médecins Sans Frontières (MSF) concluded that a viral load of 1000 copies/ ml was the level most frequently used as the trigger for clinical intervention. Furthermore, the threshold for detecting treatment failure was lowered in South Africa in April 2010 from 5000 to 1000 copies/ml and this has been implemented at various South African sites. (5, 16). WHO also updated their guidelines in June 2013 and now define

treatment failure as a persistently detectable viral load exceeding 1000 copies/ml, (8) rather than 5000 copies/ml. (17) Ideally, patients on ART for more than 6 months with a VL >1000 copies/ml should be counseled for adherence and retested three months later as per the WHO guidelines. If the VL remains >1000 copies/ml at follow-up, this may indicate treatment failure. Therefore, it is important that patients with VL ≥ 1000 copies/ml are detected by SAMBA Semi-Q. On the other hand, it is also important that individuals with low VL are not detected as such results may be due to "blips". VL blips are defined as intermittent episodes of detectable low VL (50-1000 copies), which return to undetectable without any intervention. (18, 19) This study evaluated the accuracy and performance of the SAMBA HIV-1 Semi-Q Test with a cut-off of 1000 copies/ml compared to gold standard viral load testing.

Methods

Determination of VL with SAMBA HIV-1 Semi-Q

RNA was extracted from 200 µl of plasma using the SAMBA sample-preparation instrument, SAMBAprep (Figure 1A). The result was read visually after isothermal amplification and dipstick detection within the SAMBAamp instrument (Figure 1A). The presence of a control line indicates a valid test and the test line on the dipstick indicates a VL of >1000 copies/ml, whereas the absence of the test line indicates a VL of <1000 copies/ml. The absence of both lines indicates an invalid test (Figure 1B). This assay does not detect HIV-2 and should not be used for diagnosis of HIV-1 infection.

Preparation of dilution panels of HIV-1 subtype C samples for validation of the SAMBA HIV-1 Semi-Q cut-off of 1000 copies/ml

We obtained four surplus samples from blood donors identified as positive for antibodies to HIV-1 from the National Blood Transfusion Centre in Windhoek, Namibia. Viral genotyping and VL quantification of the samples with the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v2·0 (Roche TaqMan v2) were performed at The Royal London Hospital. Serial 2-10 fold dilutions of the four samples were prepared in HIV-negative plasma to achieve HIV-1 RNA concentrations ranging from 3 (0.48 $\log_{10}$) to 222,238 (5.35 $\log_{10}$) copies/ml according to the quantification by Roche TaqMan v2. Four replicates of each dilution were tested with the SAMBA HIV-1 Semi-Q by two operators in-house to assess the precision and accuracy of the test. A total of 96 dilutions were tested.

Data analysis

The limit of accuracy for Roche TaqMan v2 is ±0.3 $\log_{10}$ relative to the VL readout obtained, according to the package insert. For the purpose of the present study, we therefore considered that any VL quantification by Roche TaqMan v2 within 0.3 $\log_{10}$ of the SAMBA HIV-1 Semi-Q cut-off of 1000 (3 $\log_{10}$) copies/ml, corresponding to a range of 500 to 2000 copies/ml, was concordant with the SAMBA HIV-1 Semi-Q result.

In-house blinded testing of clinical samples

Plasma samples from 134 HIV-1–infected individuals attending The Royal London Hospital (34 patients) or St Thomas' Hospital (100 patients) in London were rendered anonymous and provided blinded. The plasma samples were stored at –80°C until tested in-

house with the SAMBA HIV-1 Semi-Q Test. Roche TaqMan v2 results and HIV-1 subtype information (if available) were provided by both hospitals after the SAMBA HIV-1 Semi-Q testing was completed. In addition, two EQA subtype panels from Rush University RNA004XPA and RNA004XPB, containing 84 samples per panel including subtypes A, A/E, A/G, C, D, F and G at 2,500 and 25,000 copies/ml were tested blinded in-house.

Field-testing of SAMBA HIV-1 Semi-Q in Malawi and Uganda

Field-testing of the SAMBA HIV-1 Semi-Q Test was performed at ART provision program centers run by MSF in Malawi (Chiradzulu District Hospital) and Uganda (Arua Regional Referral Hospital). The Chiradzulu HIV program, which was established in 2000 and currently monitors 25,000 patients on ART, has been described in detail previously.(20, 21) The HIV program in Arua was established in 2002 and follows 7000 HIV-infected individuals on ART.(22)

Samples were collected both from consecutive patients attending the HIV clinics, on ART and pre-ART patients, in order to obtain a wide range of VLs. Plasma was separated from whole blood specimens (10 ml) within 4 hours of collection and tested fresh. In Malawi, the first 117 samples were sent to Cambridge for testing while the local technician was trained. The remaining 83 samples were tested on-site by a trained MSF technician. In Uganda, the first 120 samples collected were tested on-site by the MSF technician, and the remaining 34 samples were shipped to Cambridge for testing due to time constraints. All 354 samples from Malawi and Uganda were tested with Roche TaqMan v2 at The Royal London Hospital. In the case of discrepancy between Roche TaqMan v2 and SAMBA Semi-Q,

159 remaining frozen plasma was tested using Abbott RealTime HIV-1 Assay (Abbott
160 RealTime) by an independent laboratory.
161
162 **Analysis of VL distribution among patients in Malawi and Uganda according to**
163 **available clinical information**
164 Patient records were accessed, after consent was obtained, for the 354 patients in Malawi
165 and Uganda and were analyzed after all testing was complete. The VL of the 284 treated
166 patients was compared with that of the 70 treatment-naïve individuals in order to determine
167 the VL spread. In addition, the VL of 232 patients on ART for 0.5 to 9 years was stratified
168 according to treatment duration and used to compare the number of individuals defined as
169 virologically suppressed according either to the 2010 WHO guidelines or to the SAMBA
170 HIV-1 Semi-Q cut-off.
171
172 **Research ethics**
173 The study was performed in accordance with the Declaration of Helsinki. Ethical approval
174 was obtained from the National Health Sciences Research Committee, Ministry of Health
175 and Population, for Chiradzulu Hospital (Malawi), from the Uganda National Council for
176 Science and Technology for Arua District Hospital (Uganda), and from the Research Ethics
177 Committee, NRES-London, for St Thomas' Hospital (United Kingdom). Nucleic acid
178 testing of blood donor samples is approved in Namibia, and the four donors in the present
179 study were informed of potential additional testing. Surplus samples obtained from patients
180 known to be infected with HIV-1 and submitted to The Royal London Hospital for routine
181 monitoring were retrieved before being discarded, rendered anonymous and provided

blinded for the purpose of test validation; the use of samples in this manner, strictly for the purpose of diagnostic assay validation, does not fall under the requirements of research ethics for the organizations in which they originated.

Results

Validation of the accuracy of the SAMBA HIV-1 Semi-Q cut-off

All 52 dilutions of the four Namibian samples (all HIV-1 subtype C) containing >1000 (>3.0 $\log_{10}$) copies/ml according to Roche TaqMan v2 were correctly identified as such with SAMBA Semi-Q, and 43 of 44 (98%) dilutions containing <1000 copies/ml according to Roche TaqMan v2 were similarly correctly identified by SAMBA Semi-Q (Table 1). One of the dilutions that tested negative by SAMBA Semi-Q but according to Roche TaqMan v2 should contain 1211 (3·08 $\log_{10}$) copies/ml and two of the dilutions found to be positive by SAMBA Semi-Q that should contain 606 (2·78 $\log_{10}$) copies/ml according to Roche TaqMan v2 were considered concordant because of the accuracy limits of the TaqMan assay (see Methods). Therefore, the overall concordance between SAMBA HIV-1 Semi-Q and Roche TaqMan v2 for these 96 sample dilutions was 99% (95/96; 95% CI, 93.8–99.9).

Specificity

The specificity of the SAMBA HIV-1 assay was evaluated by testing 216 HIV-1 seronegative plasma patient specimens. The assay was not reactive for all 216 specimens and the SAMBA HIV-1 assay specificity was calculated to be 100% (216/216), (95% CI 98.6 to 100%). The specificity of the SAMBA HIV-1 Test was further evaluated using a panel of 43 specimens obtained from samples containing the following viruses, microrganisms or antibodies from autoimmune disorders: Hepatitis A (2 samples), Hepatitis B (24 samples), Hepatitis C (3 samples), CMV (2 samples) and 1 sample of each of HIV-2, HTLV I, HTLV II, Syphilis, anti-nuclear antibodies (ANA), Chlamydia

trachomatis, Neisseria gonorrhoeae, Propiobacterium acnes, Staphylococcus aureus, Candida albicans, Staphylococcus epidermis, Streptococcus pyogenes. All tested negative by SAMBA.

Potentially interfering substances

The susceptibility of the SAMBA Semi-Q test to interference by elevated levels of endogenous substances and drugs commonly prescribed to HIV-1 infected individuals was evaluated. HIV-1 negative samples and samples containing 1,000 and 2,000 IU/ml of HIV-1 RNA were tested in the presence of the following substances: Hemoglobin (500 mg/dL), Triglycerides (3000 mg/dL), Bilirubin (20 mg/dL) and Human DNA (0.4 mg/dL). No interference in the performance of the SAMBA Semi-Q test was observed. Testing of ART drugs at concentrations in excess of 1.5 times the peak plasma level (C_{max}) was performed using the following: Abacavir/Lamivudine, Efavirenz/Tenofovir/Emtricitabine, Lopinavir/Ritonavir, Lamivudine/Zidovudine, Nevirapine, Ribavirin and Saquinavir. No interference on the performance of the SAMBA Semi-Q test was observed.

In-house blinded testing of clinical samples

A total of 134 HIV-1–positive plasma samples from 100 male and 34 female patients attending two London hospitals were tested with the SAMBA HIV-1 Semi-Q Test in a blinded manner in-house. SAMBA Semi-Q was concordant with Roche TaqMan v2 for 131 of the 134 samples when four specimens (VL of 800, 948, 1285, and 1507 copies/ml respectively) were considered concordant because of the accuracy limits of the TaqMan assay (Table 2). One of 35 samples found to contain >2000 (3.3 $\log_{10}$) copies/ml by Roche

TaqMan v2 was negative by SAMBA Semi-Q (2508 copies/ml), and 2 of 95 samples found to contain <500 (2.7 $\log_{10}$) copies/ml by the Roche TaqMan assay tested positive by SAMBA Semi-Q (53 and 252 copies/ml). The concordance between SAMBA HIV-1 Semi-Q and Roche TaqMan v2 was thus 97.8% (131/134; 95% CI, 93.3–99.5). Viral subtype data provided for 76 of these samples after the SAMBA HIV-1 Semi-Q testing was completed revealed a distribution of 44.7% subtype B; 18.4% C; 10.5% CRF02_AG; 5.3% A; 2.6% CRF01_AE, F, G, G/CRF02_AG, CRF11_cpx/CRF13_cpx and A/AE; 1.3% D, CRF06_cpx, D/A and D/F. The subtypes for only two of the three discrepant samples (B and A/D) were available. In addition, a blinded EQA subtype panel provided by Rush University was tested and all 168 samples (84 at 2,500 and 84 at 25,000 copies.ml), including subtypes A, CRF01_AE, CRF02_AG, C, D, F and G, were successfully detected by SAMBA.

Field-testing of SAMBA HIV-1 Semi-Q in Malawi and Uganda

A total of 200 samples collected in Chiradzulu, Malawi, were from 72 men and 128 women, with the patients ranging in age from 18 to 61 years. Four patients were assigned an ID but withdrew from the study with no sample being collected. The 154 samples collected in Arua, Uganda, were from 68 men and 86 women, with ages ranging from 18 to 71 years. Overall, 70 patients (19.8%) were ART naïve and 284 (80.2%) had been on ART for a period of 1 month to 10 years at the time of testing.

A total of 196 of the 200 samples collected in Malawi were correctly classified by SAMBA HIV-1 Semi-Q (Table 2), with five samples (VLs of 601, 651, 922, 1539, and 1599

copies/ml) being included as concordant because of the accuracy limits of Roche TaqMan v2. For Uganda, 146 of the 154 samples were concordant between SAMBA HIV-1 Semi-Q and Roche TaqMan v2, with one sample (VL of 1061 copies/ml) being classified as such as a result of the accuracy limits of the TaqMan assay. The concordance between SAMBA HIV-1 Semi-Q and Roche TaqMan v2 was thus 96.6% overall, 98.0% in Malawi, and 94.8% in Uganda (Table 2).

Taking into consideration all data, 18 samples (5%) were discrepant between SAMBA and Roche, including six samples within the ±0.3 $\log_{10}$ of the SAMBA Semi-Q cut-off. Twelve of the 354 samples (3.4%) were truly discordant, with a VL outside of the ±0.3 $\log_{10}$ accuracy of the SAMBA Semi-Q cut-off. These 12 samples (four from Malawi and eight from Uganda) included seven found to contain <500 (2.7 $\log_{10}$) copies/ml and five found to contain >2000 (3.3 $\log_{10}$) copies/ml by Roche TaqMan v2 (Figure 2 and Table 2). These 12 discordant samples were retested with Abbott RealTime at one of two independent laboratories and in a blinded manner with regard to the SAMBA HIV-1 Semi-Q and Roche TaqMan v2 results. The Abbott RealTime results were concordant with the Roche TaqMan v2 results for 10 of the 12 samples (Figure 2). Two of the original five discrepant samples found to contain >2000 (3.3 $\log_{10}$) copies/ml by Roche TaqMan v2 were found to contain <1000 copies/ml by Abbott RealTime.

Analysis of VL distribution among African patients according to available clinical information

The VL of the 284 treated patients from Malawi and Uganda ranged from 0 to 2.6×10^6 copies/ml, whereas that of the 70 treatment-naïve individuals ranged from 1.2×10^2 to >1.0 x 10^7 copies/ml (Figure 3). In both Malawi and Uganda, the SAMBA Semi-Q cut-off value of 1000 copies/ml clearly separated individuals in the untreated group from those in the treated group.

The VL results obtained with Roche TaqMan v2 for 232 patients on ART for 0.5 to 9 years were stratified according to duration of therapy, and virological suppression was defined as either <1000 copies/ml (SAMBA HIV-1 Semi-Q cut-off) or <5000 copies/ml (2010 WHO guidelines) (Figure 4). With either definition, 93.8% of individuals manifested virological suppression after 0.5 to 1 year on treatment. The percentage of individuals with suppression fluctuated between 80.6 and 96.0% (1000 copies/ml definition) or between 83.9 and 96.0% (5000 copies/ml definition) for treatment durations of 1 to 9 years. Similar results were obtained with the two definitions of virological suppression because only six individuals (2.6%) on treatment for 0.5 to 9 years had a VL between these two values.

Discussion

The SAMBA HIV-1 Semi-Q cut-off was validated for accuracy in comparison with the Roche TaqMan v2 test with the use both of diluted clinical samples and of blinded plasma samples in-house and in the field in two public-sector ART provision programs in Africa. This study demonstrated that the SAMBA HIV-1 Semi-Q Test is able to effectively differentiate between patients with a VL above or below the defined threshold of 1000 copies/ml. The current gold-standard VL assays have a given accuracy acceptance criterion

of ±0.3 $\log_{10}$ for Roche TaqMan v2 and ±0.25 $\log_{10}$ for Abbott RealTime relative to the nominal input concentration.(23) For the present evaluations, we selected Roche TaqMan v2 as the gold standard as it is more widely used. Any samples found to contain 500 to 2000 (2.7 $\log_{10}$ to 3.3 $\log_{10}$) copies/ml by this assay were therefore considered concordant with the SAMBA result, given that the true VL might lie on either side of the cut-off. A dilution series for four samples containing HIV-1 subtype C tested by two operators revealed excellent concordance (99.0%). The subtype coverage of SAMBA was evaluated using a blinded EQA panel from Rush University, which SAMBA detected 100% of the samples including subtype A, CRF01_AE, CRF02_AG, C, D, F and G. The SAMBA HIV-1 Semi-Q cut-off was further validated in-house with a blinded panel of clinical samples, including HIV-1 subtypes A–G and a range of recombinants, yielding an accuracy of 97.8%, showing that the accuracy extends over a wide variety of viral subtypes. The reproducibility and accuracy of the SAMBA HIV-1 Semi-Q Test with fresh clinical samples collected in the field were evaluated by a trained field technician in each of two public-sector ART provision programs in Malawi and Uganda. The data were again compared with Roche TaqMan v2 results, revealing an overall concordance of 96.6%. In total, the concordance of SAMBA HIV-1 Semi-Q with Roche TaqMan v2 as determined with clinical samples from London and Africa was 97.3% (568/584; 95% confidence interval, 95.6–98.3), indicating that the performance of the SAMBA HIV-1 Semi-Q Test is in line with that of the available commercial assays. Importantly, SAMBA Semi-Q was performed on-site in Malawi and Uganda by trained MSF technicians, showing that it is simple enough to serve as an appropriate diagnostic platform for use in district hospitals in sub-Saharan Africa.

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323 Our data suggest that SAMBA HIV-1 Semi-Q, with its cut-off of 1000 (3 $\log_{10}$) copies/ml,
324 is likely to prove a useful tool for assessment of the efficacy of ART and for identifying
325 patients either who have developed virological failure and possible antiretroviral resistance
326 or who have been infected with a drug-resistant strain of HIV-1. This cut-off level should
327 also help to minimize unnecessary treatment switching due to viral blips. In addition, given
328 that the test can be performed in the field within 90 minutes, the patient can remain on-site
329 and appropriate action can be taken during the same visit. This is hugely beneficial, given
330 the fact that patients frequently face very long journeys to and from the health centers. In
331 Khayelitsha, South Africa, the ability of ART to reduce VL to an undetectable level was
332 found to correlate with the timing of viral detection and the subsequent treatment adherence
333 support provided. [4] The availability of an easy-to-use, semi-quantitative, and inexpensive
334 rapid test to detect virological failure would therefore be expected to make an important
335 contribution to optimization of first- and second-line treatment in resource constrained
336 countries. (7)

337

338 Our analysis of VL distribution in African patients indicated that the SAMBA HIV-1 Semi-
339 Q cut-off is able to reliably differentiate patients on effective ART from non-treated
340 patients as well as identifying patients with virological failure according to current WHO
341 guidelines. Analysis of the VL of 232 patients on ART for 0.5 to 9 years showed that the
342 temporal pattern for virological failure as defined in the SAMBA HIV-1 Semi-Q model and
343 current WHO guideline (>1000 copies/ml) was highly similar to that observed with the
344 2010 WHO guidelines (>5000 copies/ml).

One key advantage of the SAMBA system is that it relies on visual detection of nucleic acid on a test strip, with a readout similar to that of an HIV antibody rapid test. The processed test strip can be shown to the patient as a reinforcement tool. However, although the difference in signal strengths between positive and negative results for SAMBA HIV-1 Semi-Q is greater than that seen with many rapid tests, there remains the possibility of transcription errors or misinterpretation of results by operators in the field. This limitation will be overcome by the development of SAMBA 2, a fully integrated system in the form of a small bench-top instrument, into which the sample is introduced and the result appears on a screen or be printed on paper.

SAMBA HIV-1 Semi-Q is a semi-quantitative test for differentiation between patients with a VL above or below 1000 copies/ml, which may be regarded as a limitation of the assay given that currently available commercial tests provide a numerical readout. Although these readouts appear accurate, the accuracy of the results differs between tests, being ±0.3 $\log_{10}$ for Roche TaqMan v2 and ±0.25 $\log_{10}$ for Abbott RealTime. Furthermore, the Abbott assay consistently reads lower than the Roche test, (23) which was apparent in our analysis of discrepant samples. These VL numbers can be useful for tracking the initial virological response of individuals to treatment and are routinely reported to patients in industrialized countries. However, in LMICs, where mass treatment-monitoring is required but is currently not available, the main need of the clinician is to identify individuals who are not responding to treatment and act accordingly as soon as possible. MSF have implemented

SAMBA Semi-Q for routine use at one site in Arua and two sites in Chiradzulu since August 2013. Patients are monitored twice per year and results are given at the same visit. Those with a VL >1000 copies/ml are counseled for adherence reinforcement and if they still have a viral load >1000 copies/ml at the next visit they are switched to second-line therapy.

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461 Table 1. Validation of the SAMBA HIV-1 Semi-Q cut-off with diluted plasma samples
462 containing HIV-1 subtype C.
463

Sample	HIV-1 RNA (copies/ml)	Positive SAMBA HIV-1 Semi-Q result (>1,000 copies/ml)
1	151,114	100%
	15,111	100%
	1511	100%
	756	0%
	151	0%
	15	0%
2	30,543	100%
	3054	100%
	1527	100%
	305	0%
	31	0%
	3	0%
3	222,238	100%
	22,224	100%
	2222	100%
	1111	100%
	222	25%

	22	0%
4	121,102	100%
	12,110	100%
	1211	75%
	606	50%
	121	0%
	12	0%

Four plasma samples were serially diluted to achieve concentrations of viral RNA ranging from 3 (0·48 $\log_{10}$) to 222,238 (5·35 $\log_{10}$) copies/ml according to quantification with Roche TaqMan v2. Four replicates of each dilution were tested with SAMBA HIV-1 Semi-Q.

469 Table 2. Comparison of SAMBA HIV-1 Semi-Q and Roche TaqMan v2 results for 488
470 clinical samples from London (*n*=134), Malawi (*n* =200) and Uganda (*n* = 154).
471

SAMBA Semi-Q (copies/ml)	**Roche TaqMan v2 (copies/ml)**	
London	**<1000**	**>1000**
<1000	95	1
>1000	2	36
Malawi		
<1000	146	0
>1000	4	50
Uganda		
<1000	91	5
>1000	3	55

472
473 Concordance between the two tests was 96.9% (473/488) overall, 97.8% (131/134) in
474 London, 98.0% (196/200) in Malawi, and 94.8% (146/154) in Uganda.
475
476

Figure 1: SAMBA I system

A. SAMBA I instruments, SAMBAprep (right) and SAMBAamp (left) instruments

B. SAMBAamp cartridge showing results for (i) >1,000 copies/ml, (ii) < 1,000 copies/ml and (iii) invalid.

Figure 2. Field-testing algorithm for SAMBA HIV-1 Semi-Q with 354 samples collected in Malawi and Uganda and summary of results. All samples were tested with SAMBA HIV-1 Semi-Q and Roche TaqMan v2. Twelve samples were discrepant between SAMBA and Roche and were tested with Abbott RealTime. Ten of the twelve samples were discrepant between SAMBA and Abbott, two were discrepant between Abbott and Roche.

Figure 3. Distribution of VL among 284 patients receiving ART and 70 untreated individuals in Malawi and Uganda. VL was determined with Roche TaqMan v2.

Figure 4. Virological suppression according to the SAMBA HIV-1 Semi-Q cut-off and 2013 WHO guidelines (1000 copies/ml) or 2010 WHO guidelines (5000 copies/ml) in 232 patients on ART for various number of years in Uganda and Malawi.