WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: SAMBA II HIV-1 Qual Whole Blood Test
WHO reference number: PQDx 0458-072-00

SAMBA II HIV-1 Qual Whole Blood Test with product code 4500-12, manufactured by Diagnostics for the Real World Ltd**,** CE-mark regulatory version, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 19 July 2023.

Summary of WHO prequalification assessment for SAMBA II HIV-1 Qual Whole Blood Test

	Date	Outcome
Prequalification listing	19 July 2023	listed
Dossier review	21 -22 November 2019	N/A
Site inspection(s) of the quality management system	21 November 2019	MR
Product performance evaluation	Quarter 3 2022	MR

MR: Meet Requirements
N/A: Not Applicable

Intended use:

According to the claim of intended use from the Diagnostics for the Real World Ltd, " *The SAMBA (Simple Amplification Based Assay) II HIV-1 Qual Whole Blood Test is an in vitro nucleic acid-based amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) in human whole blood. The SAMBA II HIV-1 Qual Test is intended for use in clinical or point-of-care settings using the fully automated SAMBA II Assay Module (P/N I19-0006-AM) and a control unit- the SAMBA II Tablet Module (I19-0006-TM). The SAMBA II HIV-1 Qual Whole Blood Test is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult individuals. The SAMBA II HIV-1 Qual Whole Blood Test is not intended to be used as a donor screening test for HIV-1 nor for viral load monitoring in HIV-infected individuals."*

Assay description:

According to the claim of assay description from the Diagnostics for the Real World Ltd*, "The SAMBA II HIV-1 Qual Whole Blood Test is a fully automated assay run on the SAMBA II*

instrument system consisting of the SAMBA II Assay Module, and a control unit – the SAMBA II Tablet Module. Nucleic acid extraction, amplification of the nucleic acid target and the detection of the amplification products are performed in the SAMBA II Assay Module. The extraction phase of the assay involves the lysis of cells (in case of whole blood specimens) and the virus, and the release of nucleic acid into solution, which is then captured by a silica membrane column. The bound nucleic acid is washed and eluted from the membrane and the HIV target sequence is amplified in the sealed SAMBA II QB II Cartridge 1. After amplification, a colored-labeled anti-hapten detection solution is mixed with the amplification product and the mixture is wicked in a Test Strip. The test result (i.e. bluish to purple lines on the Control Line and/or Test Line) is captured by a built-in camera, which is recorded and can be read on the Tablet Module. Results are stored on the tablet, and can also be sent via SMS text. Results can also be printed from a Bluetooth printer that connects to the Tablet Module."

Test kit contents:

Component	**12 tests (product code 4500-12)**
QB II Cartridge 1 (PN: 4500A)	1
QB II Cartridge 2 (PN: 4500B)	1
QB II Cartridge 3 (PN: 4500C)	1
QB II Cartridge 4 (PN: 4500D)	1

Items required but not provided:

Equipment:

- SAMBA II Assay Module (I19-0006-AM),
- SAMBA II Tablet Module (I19-0006-TM).

Materials required and provided as accessories to the kit:

- Sample Collection Tube, EDTA (KABE Code No. 07 6014) or Sample Collection Tube, EDTA, 12 pack (C13-0001-12),
- Alcohol swabs (C01-0005)
- Lancet (C12-0005)
- SAMBA II Sample card, QB (C19-0045) OR SAMBA II Blood Collection Kit (C19-0065)

Other materials available to order as accessories to the kit:

- Sample Collection Tube, Untreated (KABE Code No. 05 3615) or
- Sample Collection Tube, Untreated, 12 pack (C19-0015-12) (only required for samples collected by Venipuncture).

Storage:
The test kit should be stored at 2-37 °C.

Shelf-life upon manufacture:
9 months.

Warnings/limitations:
Please see attached the IFU attached to this public report.

Prioritisation for prequalification:
Based on the established criteria, SAMBA II HIV-1 Qual Whole Blood Test was given priority for WHO prequalification assessment.

Product dossier assessment

In accordance with the WHO procedure for abridged prequalification assessment, Diagnostics for the Real World Ltd was not required to submit a product dossier for SAMBA II HIV-1 Qual Whole Blood Test as per the *"Instructions for compilation of a product dossier"* (PQDx_018 version 3). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Manufacturing site inspection

An onsite inspection of Diagnostics for the Real World in San Jose, CA, USA, was conducted on 21 and 22 November 2019. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality.

Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for in vitro diagnostic products and gives a summary of the inspection findings.

Information on the most current inspection can be found at:
https://extranet.who.int/pqweb/inspection-services/prequalification-reports/whopirs-vitro-diagnostics

All published WHOPIRs are with the agreement of the manufacturer.

Based on the site inspection and corrective action plan review, the quality management system for SAMBA II HIV-1 Qual Whole Blood Test meets WHO prequalification requirements.

Product performance evaluation

SAMBA II HIV-1 Qual Whole Blood Test (Diagnostics for the Real World Ltd) was evaluated by the Central Public Health Laboratories, Kampala, Uganda, on behalf of WHO in the 3rd quarter of 2022, according to protocol PQDx_199 v2.0.

Clinical performance evaluation

In this limited laboratory-based evaluation of clinical performance characteristics, a panel of 520 whole blood specimens was used. The specimens were characterised using the following reference assay: COBAS AmpliPrep/COBAS TaqMan HIV-1 Qualitative Test, version 2.0 (Roche Diagnostics).

Clinical performance characteristics in comparison with an agreed reference standard		
	Infants < 18 months	**Adults**
Sensitivity (N=115 infants / 98 adults)	98.3% (95% CI: 93.9% - 99.8%)	98.0% (95% CI: 92.8% - 99.8%)
Specificity % (N= 199 infants / 99 adults)	100% (95% CI: 98.2% - 100%)	99.0% (95% CI: 94.5% - 100%)
Invalid/error rate % (N= 520)	3.8%	

Analytical performance evaluation

Analytical performance characteristics	
Limit of detection (LoD) using the 4th WHO International Standard for HIV-1 RNA – whole blood	The LoD for HIV-1 was estimated at 4871 IU/mL (95% CI: 2921-8124 IU/mL). The LoD claimed by the manufacturer (1909 IU/mL, 95% CI: 1391-3008 IU/mL) was verified.
Limit of detection (LoD) using the VQA Standard for HIV-1 RNA	The LoD was estimated at 1535 copies/mL (95% CI: 901-2616 copies/mL). The LoD claimed by the manufacturer (742 copies/mL; 95% CI: 503-1329 copies/mL) was verified.

Within-laboratory precision (reproducibility)	The hit rate for HIV-1 subtype B at 1330 cp/mL, was 100% (32/32). The hit rate for HIV-1 subtype C at 1330 cp/mL was 100% (33/33).
Subtype detection	HIV-1 subtypes B, C, D, F, AE and AG at 5000 cp/mL were detected.
Cross-contamination / carry-over	No carry-over was observed when high positive and negative specimens were tested alternatively.

Operational characteristics and ease of use

This assay does not require laboratory equipment outside of the SAMBA II instrument and tablet and can be performed in laboratories with limited facilities or in point-of-care settings. The instrument is supplied with an uninterruptible power supply (UPS).

The assay was found easy to use by the operators performing the evaluation, who had received a one-day training from the manufacturer prior to the evaluation.

Key operational characteristics	
Specimen type(s) and volume	140 µl of venous or capillary whole blood
Number of steps for one specimen*	6 steps in total 1 steps with precision pipetting
Number of steps for instrument management**	1 step per day
Time to result for one test/run	120 to 135 minutes
Operator hands-on time for one test	5 minutes
Level of automation	Fully automated
Quality controls	Each test is provided with an Internal Control. QC is not provided by the manufacturer.
Operating temperature	10-38 °C
Result display and connectivity	Results are displayed on the connected tablet. They may be printed using a specific printer (SAMBA II Printer Module, P/N C16-0032). The results can be exported to the laboratory information system and other health information systems.

Power sources	Main power. The use of a UPS is recommended, as stable electricity is required.
Biosafety *(outside of infectious specimen handling)*	The QBII cartridge 2 contains ProClin, which may cause an allergic skin reaction, causes skin irritation, causes serious eye irritation.
Waste	The volume of liquid is approximately 2mL per test/run. Solid waste volume is approximately 81.0 g per test/ run. Waste disposal does not require specific measures in addition to usual laboratory biohazard waste disposal procedures.
Calibration	Calibrators are not provided by the manufacturer and should be purchased separately. Calibration is only needed after annual preventive maintenance or major breakdown.
Maintenance	Minimal maintenance (clean filter), daily cleaning is recommended.

** Steps for one specimen: each action required to obtain a result for one specimen (excluding specimen collection, instrument management, maintenance/calibration), e.g. add specimen to the cartridge, close the cartridge, scan/type specimen ID, load the cartridge on the instrument, press start (5 steps) OR scan/type specimen ID, load the specimen collection tube into the instrument, press start (3 step)*

*** Steps for instrument management: each action required daily or per run to set up and shut down the instrument, e. g. switch on instrument, login, maintain supplies, maintain reagents, discard liquid waste, discard solid waste, archive results, switch off instrument (8 steps)*

Based on these results, the performance evaluation for SAMBA II HIV-1 Qual Whole Blood Test meets the WHO prequalification requirements.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

1.1 Packaging box label

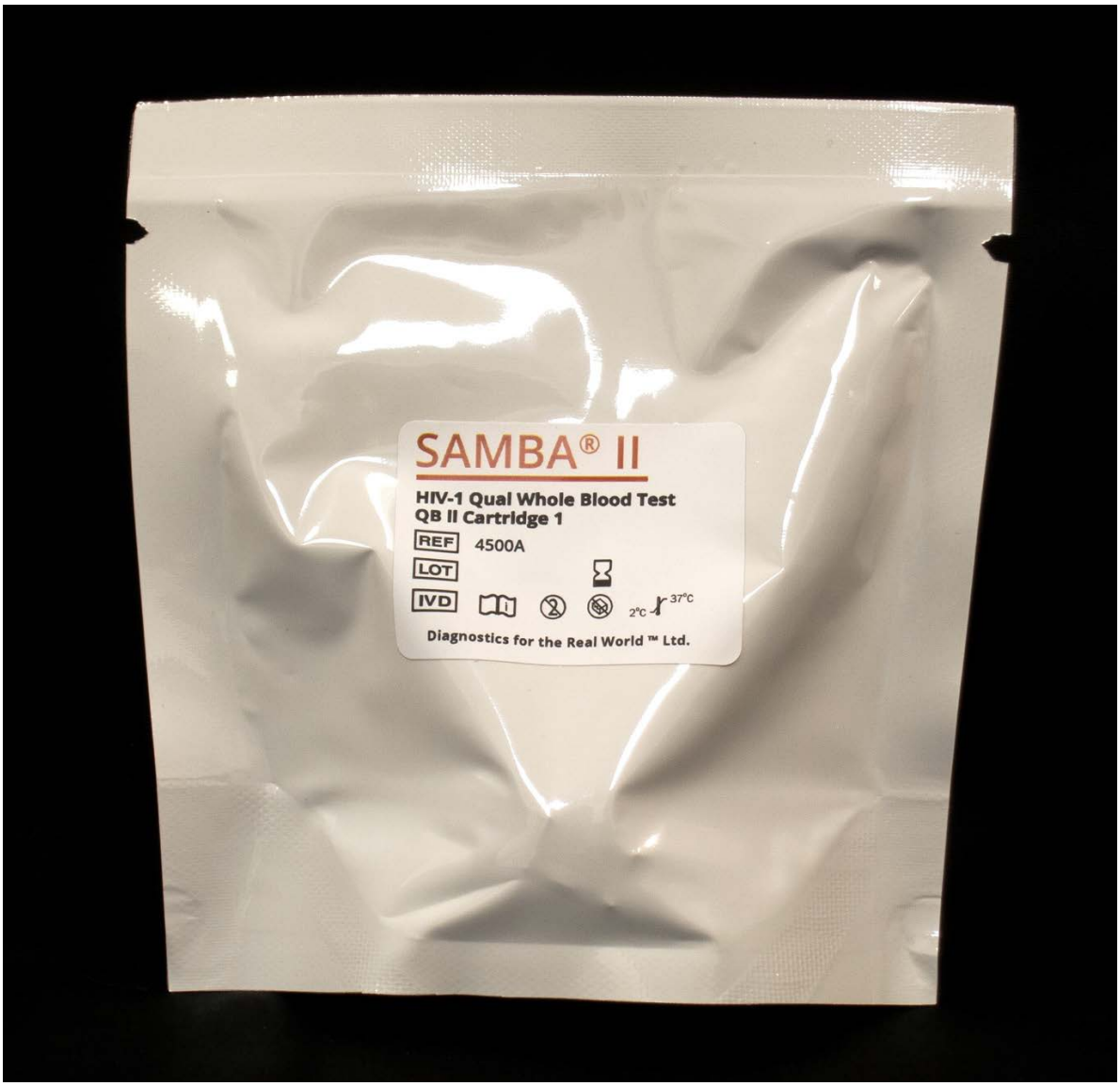
Front

Back


DRW
Diagnostics for the Real World
Diagnostics for the Real World™ Ltd.
845 Embedded Way, Suite 150, San Jose, CA 95138 USA

1.2 Test set bag (Code No: 4500)

1.3 QB II Cartridge 1 (Code No: 4500A), In pouch



1.4 QB II Cartridge 3 (Code No: 4500C) – In pouch

2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Diagnostics for the Real World

SAMBA II HIV-1 Qual Whole Blood Test

(Simple nucleic acid AMplification Based Assay)

in vitro test

REF 4500-12

C08-0008-EN V13.0 Approved: 27 Jul 23

INSTRUCTIONS FOR USE

Read these Instructions for Use carefully prior to use. Perform the test in accordance with the instructions to ensure reliability of assay results. Do not deviate from the instructions prescribed in these Instructions for Use.

NAME

SAMBA II HIV-1 Qual Whole Blood Test

INTENDED USE

The SAMBA (Simple Amplification Based Assay) II HIV-1 Qual Whole Blood Test is an *in vitro* nucleic acid-based amplification assay for the qualitative detection of Human Immunodeficiency Virus Type 1 (HIV-1) in human whole blood. The SAMBA II HIV-1 Qual Test is intended for use in clinical or point-of-care settings using the fully automated SAMBA II Assay Module (P/N I19-0006-AM) and a control unit- the SAMBA II Tablet Module (I19-0006-TM). The SAMBA II HIV-1 Qual Whole Blood Test is intended to be used as an aid in the diagnosis of HIV-1 infection in pediatric and adult individuals. The SAMBA II HIV-1 Qual Whole Blood Test is not intended to be used as a donor screening test for HIV-1 nor for viral load monitoring in HIV-infected individuals.

SUMMARY AND EXPLANATION OF THE TEST

Current molecular tests used for diagnosis of HIV infection in exposed infants are performed in centralized laboratories using dried blood spot (DBS) specimens. Because these centralized laboratories are usually located in urban centers and far from peripheral clinics, transport of DBS samples continues to be a challenge, resulting in delays in testing, long turnaround time and loss to follow up. In adults, early infection during the “window” period when anti-HIV antibodies are not yet detectable may be inaccurately diagnosed using routine lateral flow immunoassays. In such cases, the SAMBA II HIV-1 Qual Whole Blood Test may be used to aid in the diagnosis or confirmation of early HIV infection at point-of-care settings.

Because of the need for testing HIV infection as close to the patient as possible, DRW developed the SAMBA II HIV-1 Qual Whole Blood Test, a qualitative assay for detection of HIV-1 RNA and DNA in whole blood specimens.

PRINCIPLES OF THE TEST

The SAMBA II HIV-1 Qual Whole Blood Test is a fully automated assay run on the SAMBA II instrument system consisting of the SAMBA II Assay Module, and a control unit – the SAMBA II Tablet Module. Nucleic acid extraction, amplification of the nucleic acid target and the detection of the amplification products are performed in the SAMBA II Assay Module[1]. The extraction phase of the assay involves the lysis of cells (in case of whole blood specimens) and the virus, and the release of nucleic acid into solution, which is then captured by a silica membrane column[2]. The bound nucleic acid is washed and eluted from the membrane and the HIV target sequence is amplified in the sealed SAMBA II QB II Cartridge 1. After amplification, a colored-labeled anti-hapten detection solution is mixed with the amplification product and the mixture is wicked in a Test Strip. The test result (i.e. bluish to purple lines on the Control Line and/or Test Line) is captured by a built-in camera, which is recorded and can be read on the Tablet Module. Results are stored on the tablet, and can also be sent via SMS text. Results can also be printed from a Bluetooth printer that connects to the Tablet Module.

PREVENTION OF NUCLEIC ACID CONTAMINATION

Nucleic acid amplification-based assays are extremely sensitive and therefore are at risk of getting false positive results when contamination occurs. For this reason, SAMBA reagents are pre-dispensed into single-use cartridges and do not require user manipulation except at the initial step of loading the cartridges and the sample tube into each position in the Assay Module. It is highly recommended to the user to read the care and maintenance instructions for the SAMBA instrument system and observe good laboratory practice during SAMBA testing and disposal of used test materials.

MATERIALS AND REAGENTS

SAMBA II HIV-1 Qual Whole Blood Test Kit (List No. 4500-12)

NOTE: All cartridges required to test one sample using SAMBA II Qual Whole Blood Test are packaged in a One Test Set bag. Each Kit contains 12 x One Test Set bag, sufficient to test 12 samples. All cartridges are for single-use only.

Each One Test Set (List No. 4500) contains:

4500A 1 each QB II Cartridge 1

Each QB II Cartridge 1 contains a lyophilized sphere consisting of AMV reverse transcriptase, recombinant ribonuclease H, recombinant T7 RNA polymerase and a Test Strip lined with synthetic oligonucleotides specific to HIV-1 (Test Line) and a non-competitive control target (Internal Control Line). QB II Cartridge 1 is for single-use only.

4500B 1 each QB II Cartridge 2

Each QB II Cartridge 2 contains tubes with the extraction buffers for HIV-1 nucleic acids from whole blood and the detection buffer containing a mixture of salts, treated casein, 0.10% ProClin 300 and 0.15% ProClin 950. QB II Cartridge 2 also contains the disposable adapter. QB II Cartridge 2 is for single-use only.

4500C 1 each QB II Cartridge 3

Each QB II Cartridge 3 contains the lyophilized Internal Control, enzymes, anti-hapten monoclonal antibody conjugated to colloidal gold, molecular biology grade lubricant, a filter column and a lyophilized Reagent sphere which contains a mixture of dNTPs, oligonucleotide primers, synthetic oligonucleotides and salts. QB II Cartridge 3 is for single-use only.

4500D 1 each QB II Cartridge 4

Each QB II Cartridge 4 contains pipette tips and syringe components. QB II Cartridge 4 is for single-use only.

Equipment required

I19-0006-AM SAMBA II Assay Module
I19-0006-TM SAMBA II Tablet Module

Materials required and provided as accessories to the kit:

Sample Collection Tube, EDTA (KABE Code No. 07 6014) or
Sample Collection Tube, EDTA, 12 pack (C13-0001-12)

Alcohol swabs (C01-0005)
Lancet (C12-0005)
SAMBA II Sample card, QB (C19-0045)
OR
SAMBA II Blood Collection Kit (C19-0065)

Other materials available to order as accessories to the kit:
Sample Collection Tube, Untreated (KABE Code No. 05 3615) or
Sample Collection Tube, Untreated, 12 pack (C19-0015-12)
(only required for samples collected by Venipuncture)

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only

Safety Precautions

Refer to the SAMBA II Assay Module and SAMBA II Tablet Module User Guide, for instructions on safety precautions related to instruments.

- *The SAMBA II HIV-1 Qual Whole Blood Test (P/N 4500) is for use with **whole blood** collected venipuncture or using the specified accessory SAFE-T-FILL Mini capillary blood collection tube.*
- *Blood sample collected using venipuncture should be transferred to a red top Sample Collection Tube, Untreated (KABE P/N 05 3615 or C19-0015-12) with the red lid.*
- *Capillary blood from heel or finger prick should be collected into the Sample Collection Tube, EDTA (KABE P/N 07 6014 or C13-0001-12) with the purple lid provided as an accessory to the kit.*
- *Handle all specimens as though capable of transmitting infectious agents.*
- *Wear disposable powder-free gloves, lab coat and safety glasses at all times while performing the test. Change gloves as often as possible.*
- *Do not re-use the single-use kit components (e.g. cartridges) under any circumstances.*
- *Do not mix kit components from different lots.*
- *Read these Instructions for Use and the SAMBA II instrument system completely before using this product and follow the instructions carefully.*
- *Do not use kit or its components after stated expiration dates.*
- *Dispose of all contaminated materials such as cartridges and tips in accordance with local, state and federal or national regulations.*
- *Do not inhale or swallow any liquid or dry reagents. Avoid contact with skin. Rinse with plenty of water if contact occurs.*
- *Adhere to Good Laboratory Practice (GLP) while running the tests or handling specimens at all times.*
- *Do NOT attempt to open **cartridges** after use. This can lead to contamination of the laboratory with amplification products.*
- *Store the SAMBA II HIV-1 Qualitative Test kits at 2-37^{o}C*

- *Clean and disinfect all work surfaces thoroughly with Distel or any other nucleic acid cleaning agent. Freshly prepared 0.5% hypochlorite solution may also be used to clean surfaces but NOT the instruments.*

SAMBA II HIV-1 Qual **QB II Cartridge 2** contains a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, which are components of ProClin. These components are classified per applicable European Community Directives as:

Warning: (see Safety Data Sheet (SDS) for additional details)

Hazard statements:

- *H317: May cause an allergic skin reaction*
- *H315: Causes skin irritation*
- *H319: Causes serious eye irritation*

Precautionary Statements Prevention

- *P261: Avoid breathing dust/fume/gas/mist/vapors/ spray*
- *P272: Contaminated work clothing should not be allowed out of the workplace*
- *P280: Wear protective gloves/protective clothing/eye protection/face protection*
- *P264: Wash hands thoroughly after handling*

Precautionary Statements Response

- *P305+P351+P338: If in eyes, rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do; continue rinsing.*
- *P337+P313: If eye irritation persists, get medical advice/attention.*
- *P302+P352: If on skin, wash with plenty of soap and water.*
- *P362, P363: Take off contaminated clothing and wash before reuse.*
- *P333+P313: If skin irritation or rash occurs, get medical advice/attention.*

Reagent storage and handling instructions

- *Store the kit at 2-37°C*
- *Keep all cartridges in original packaging until ready for use*
- *Do not use kit components that show signs of tear or breach of packaging*
- *Do not use the kit beyond the expiration date printed on the outside of the box*
- *Do not mix kit components from different lots.*

INSTRUMENT REQUIREMENTS

The SAMBA II HIV-1 Qual **Whole Blood** Test is performed using the SAMBA II instrument system consisting of the SAMBA II Assay Module (P/N I19-0006-AM) and the SAMBA II Tablet Module *(*P/N I19-0006-TM). The SAMBA II instrument system must be set up properly prior to performing the assay. Refer to the appropriate sections on specimen handling and assay procedure described in this document and User Guide of each instrument for detailed operating procedures.

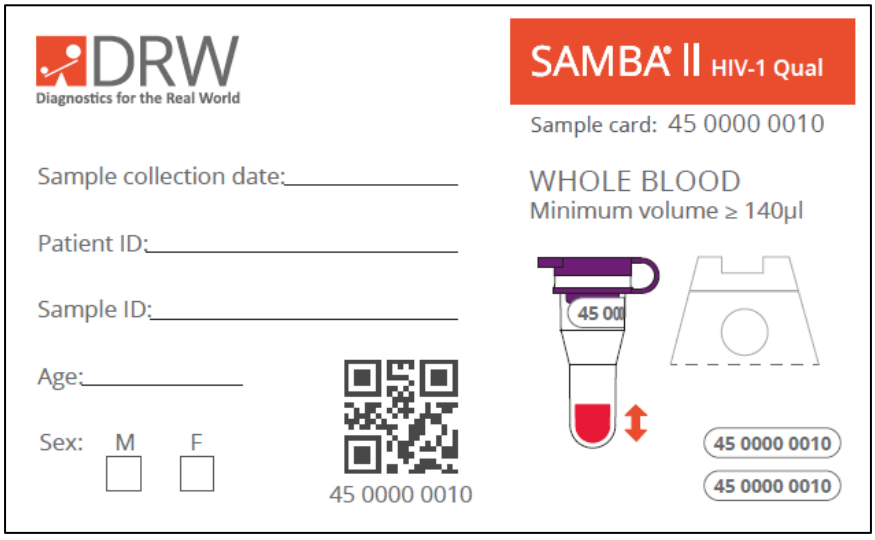
SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS

Human whole blood (K_2 EDTA, K_3 EDTA and sodium citrate) specimens may be used with the DRW SAMBA HIV-1 Qual Test. Follow the manufacturer's instructions on venipuncture collection of whole blood samples using an evacuated tube system. Transfer specimens into the Sample Collection Tube, Untreated (KABE P/N 05 3615 or C19-0015-12). For capillary finger/heel prick collection use Sample Collection Tube, EDTA (KABE P/N 07 6014 or C13-0001-12). Whole blood specimen volume in the tube should be at least 140 µl.

Freshly drawn whole blood may be held at 2-30°C for up to 18 hours prior to testing. Freezing of samples should be avoided. For refrigerated whole blood specimens, allow the sample to equilibrate to room temperature prior to testing.

INSTRUCTIONS ON THE USE OF THE SAMBA II SAMPLE CARD, QB II:

- *Fill in information required on the SAMBA II Sample Card, QB (List No. C19-0045, specific for SAMBA II HIV-1 Qual Whole Blood Test).*
- *Peel off one of the sticker labels from the SAMBA II Sample Card and attach to the Sample Collection Tube.*
- *Peel off the other sticker label from the SAMBA II Sample Card and attach to the Patient Notes*



ASSAY PROCEDURE

- *READ these instructions for use and the SAMBA II instrument system User Guides carefully before performing the test.*
- *The SAMBA II HIV-1 Qual Whole Blood Test is intended to be used only with the SAMBA II instrument system.*
- *Do not use kits beyond kit expiration date.*
- *Components within a kit are intended to be used together.*
- *Do not mix kit components from different lots.*
- *If necessary, bring the specimens to room temperature (15-30°C) before commencing the assay.*
- *To reduce the risk of nucleic acid contamination, clean and disinfect spills of specimens and reagents by using a detergent solution, followed by a tuberculocidal disinfectant such as 0.5% hypochlorite solution or other suitable disinfectant. For the instruments, follow the care and maintenance procedures before and after use detailed in the User Guide for the SAMBA II Assay Module.*
- *A manual record of the test results must be kept against patient records in accordance with the established protocols of your organization or institution.*

Additional notes:

Set-up the SAMBA II instrument system before starting the assay:

1. Switch on the SAMBA II Assay Module.
2. Switch on the SAMBA II Tablet Module.

For detailed instructions on how to operate the SAMBA II Assay Module and the SAMBA II Tablet Module, refer to respective User Guide for each instrument prior to use. Laboratory personnel must be trained to operate the SAMBA II instrument system and must follow good laboratory practice. Refer to the **WARNINGS AND PRECAUTIONS in this document prior to preparing specimens.**

A. SAMPLE PREPARATION

Prepare the whole blood sample.

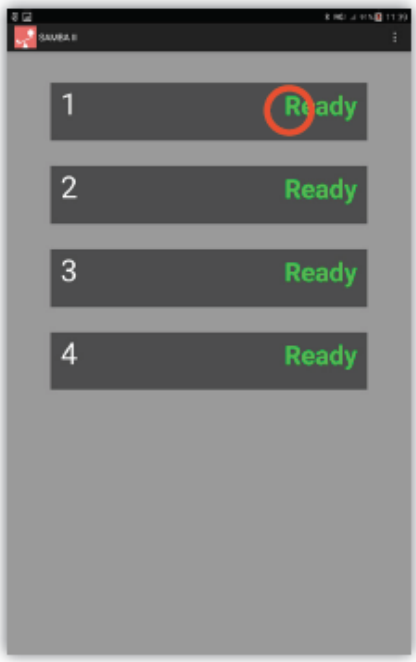
1. Put on clean, powder-free gloves.
2. Carefully remove capillary tube from the sample collection tube, EDTA and dispose capillary tube as infectious waste material.
3. Visually inspect the blood tube to ensure that there is sufficient volume of sample in the tube (140 µl minimum).

B. RUNNING THE ASSAY USING THE SAMBA II INSTRUMENT SYSTEM

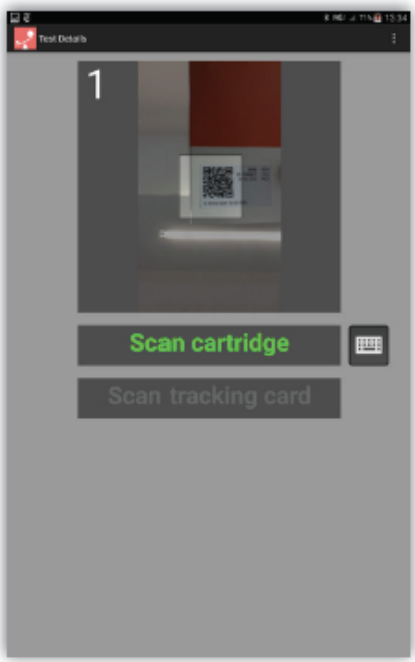
1. Switch on Tablet and unlock using the PIN or password set for device (Refer to User Guide for Tablet Module for detailed instructions- Doc no C19-0032). Locate the SAMBA II App icon on the Home screen. Tap the SAMBA II icon to launch the App.

2. App will open and display the main Application screen. When app launches, it will attempt to connect to all previously connected Assay Modules.

3. If an Assay Module is available, the indicator for the instrument will change from **Connected** to **Ready**. Select button for the Assay Module to proceed with testing.

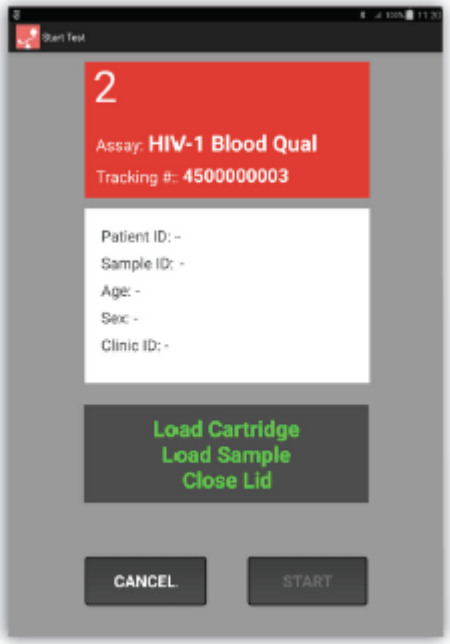


4. The **Scan Cartridge** screen appears. When the camera has been activated, the barcode on the cartridge bag can be scanned. **Scan Cartridge** will be highlighted. Scan the barcode (see SAMBA II Tablet Module User Guide for details).



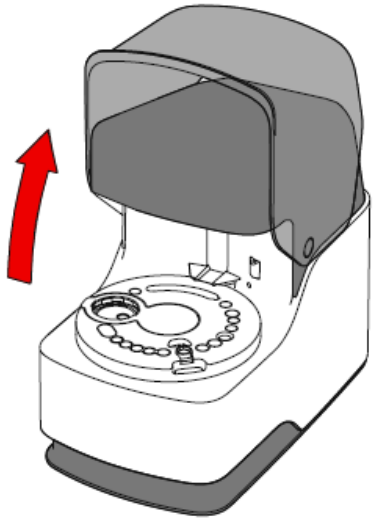
5. When the barcode is successfully registered, the Tablet will sound an alert as confirmation and **Scan Tracking Card** will be highlighted. The patient sample card must now be scanned. (Refer to Tablet Module User Guide, Doc No C19-0032 if barcode cannot be scanned.)
6. After the barcode is successfully scanned or manually entered the Tablet will sound an alert and the **Test Details** screen will appear. Additional details can be entered. After additional details are entered, the

screen prompts to **Load Cartridge-Load Sample-Close Lid.** (See the User Guide for Assay Module, Doc No. C19-0030, for details on how to load the cartridges and samples).

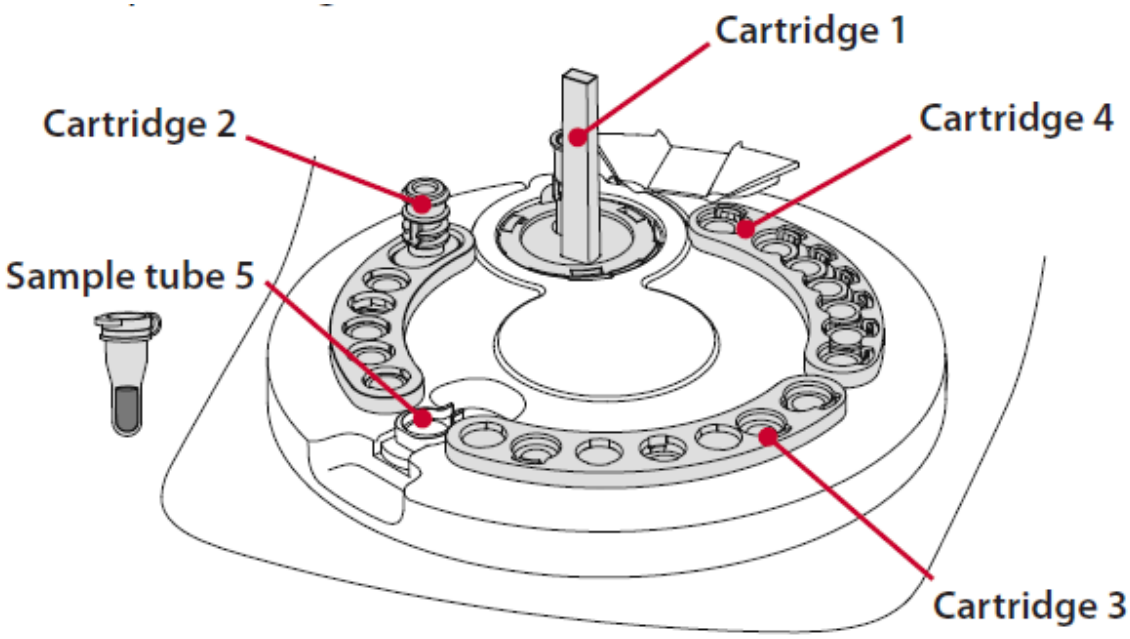


C. Set up the SAMBA II Assay Module.

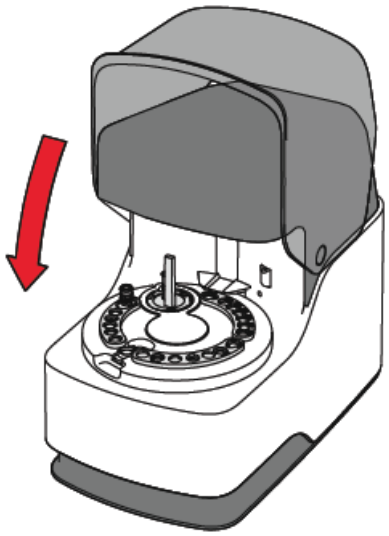
1. Set up the SAMBA II HIV-1 Qual Whole Blood Test Cartridges on the SAMBA II Assay Module.
2. Tear open the One Test Set bag and visually inspect that there are **four (4)** cartridges.
3. Confirm the contents:
   - 1 each QB II Cartridge 1 (List no. 4500A)
   - 1 each QB II Cartridge 2 (List no. 4500B)
   - 1 each QB II Cartridge 3 (List no. 4500C)
   - 1 each QB II Cartridge 4 (List no. 4500D)
4. Open the lid of the SAMBA II Assay Module by lifting it up then release. The door will stay open unaided.



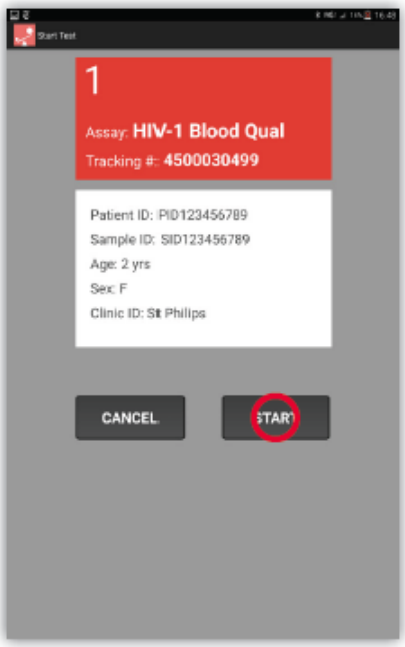
5. Load cartridges into the Assay Module following the diagram below:
   - *Remove QB II Cartridge 1 from its foil pouch and insert cartridge into position 1.*
   - *Load QB II Cartridge 2 into position 2.*
   - *Take out QB II Cartridge 3 from its foil pouch and insert into position 3.*
   - *Insert QB II Cartridge 4 into position 4.*
   - *Load the whole blood sample in the sample holder (see 5 in the figure below):*



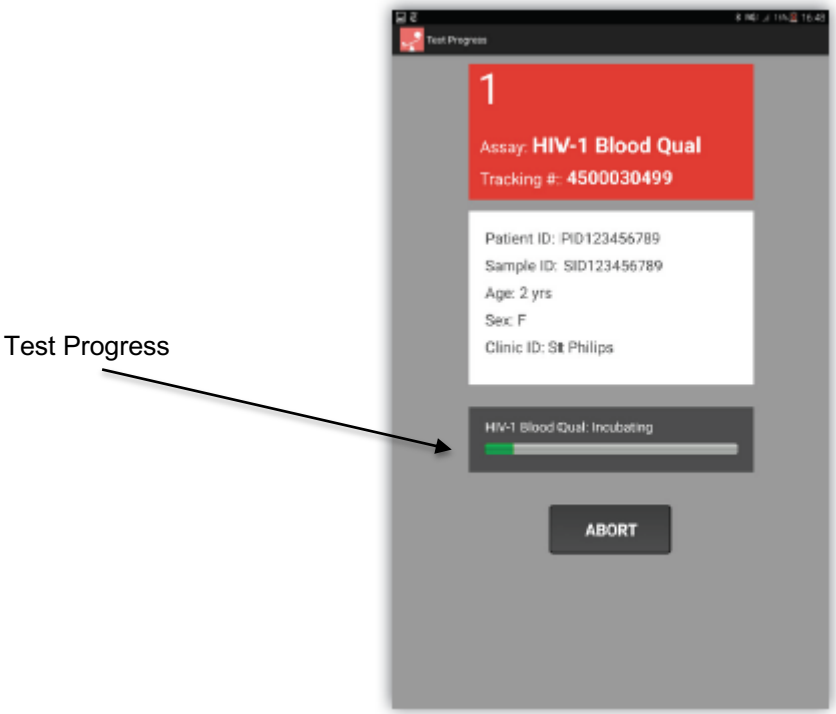
6. Ensure that all the cartridges are snugly fit in each position.
7. After the sample tube and cartridges have been loaded, close the lid of the Assay Module.



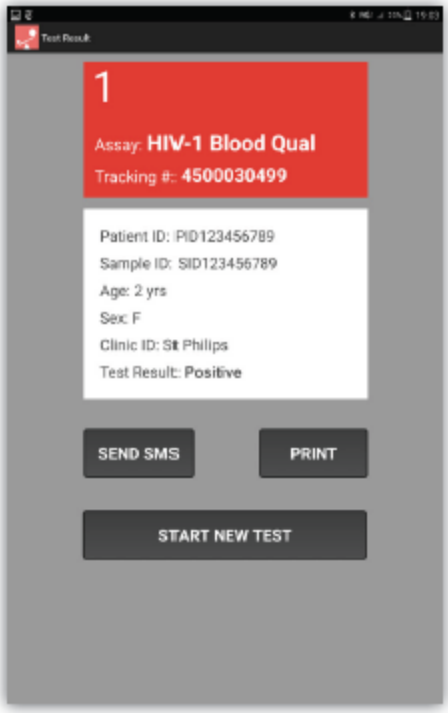
8. The start button will now be available on the Tablet Module. Press the Start button to proceed.



9. Once the test has started, a Test Progress bar will appear on the screen. The stripe on the front of the Assay Module will change from white to green. The details for the test running will appear on the Test Progress screen on the Tablet Module. The assay will run for approximately 2 hrs.



10. At the end of the test, a summary of the test details and results are displayed on the **Test Results** screen.



11. The Test Result screen also has 3 buttons- **SEND SMS, PRINT and START NEW TEST**. Refer to Tablet Module User Guide (Doc No. C19-0032) for details on sending SMS text or connecting to Bluetooth printer to print results).
12. **Note:** A manual record of the test results must be kept against patient records in accordance with the

established protocols of your organization or institution.

13. Open the lid of the Assay Module and remove used Cartridges and Sample Tube. Discard as clinical waste following routine laboratory procedures for handling infectious waste materials.

INTERPRETATION OF RESULTS

At the end of the assay, one of the following five (5) results appear on the SAMBA II Tablet Module:

- A "**Positive**" result indicates that HIV-1 was detected.
- A "**Negative**" result indicates that HIV-1 was **not** detected.
- An "**Invalid**" result indicates that the test needs to be repeated using a fresh sample.
- A "**Halted**" result indicates that the test has failed and needs to be repeated using a fresh sample.
- A "**Read Failure**" result indicates the camera has failed to read the result and the test is invalid. The test needs to be repeated using a fresh sample.

QUALITY CONTROL

Each test is provided with Internal Control, which controls for sample extraction, amplification and detection. The **Positive** or **Negative** result displayed on the SAMBA II Tablet Module indicates that the Internal Control was functioning properly. An **Invalid** result indicates that the Internal Control failed, thus the assay needs to be repeated on a fresh sample.

Limitations of the Test

For *in vitro* diagnostic use only

The SAMBA II HIV-1 Qual Whole Blood Test has been validated for the qualitative detection of HIV-1 nucleic acids (RNA/proviral DNA in human whole blood). Optimal performance of these tests require appropriate specimen collection, handling and storage as described in the Specimen Collection and Handling section of this Instructions for Use.

SAMBA II HIV-1 Qual Whole Blood Test has been validated on whole blood specimens only; it has not been validated for dry blood spot (DBS). The SAMBA II Qual Whole Blood Test reagents and procedures should be run in conjunction with the SAMBA II instrument system. Other sample preparation and amplification instruments have not been validated with the SAMBA II HIV-1 Qual Whole Blood Test.

The SAMBA II HIV-1 Qual Whole Blood Test requires personnel trained on the SAMBA II instrument system to run the instrument and the assay procedures.

The SAMBA II Qual Whole Blood Test procedures and instrument system have been designed to reduce the risk of contamination with amplification product. However, contamination from specimens and other sources must be prevented by good laboratory practice and strict adherence to the procedures described in the Instructions for Use.

As with any other *in vitro* diagnostic test, the qualitative detection of HIV-1 is dependent on several factors such as the number of virus present in the specimen, the quality of the specimen, etc. Results from the SAMBA II Qual Whole Blood Test must be interpreted in conjunction with other clinical symptoms and laboratory findings.

A specimen result of “HIV-1 RNA/DNA not detected” cannot be presumed to be negative for HIV-1.

As with any other in vitro diagnostic test, the detection of HIV-1 is dependent on several factors such as the number of virus present in the specimen, the quality of the specimen, etc. Results from the SAMBA I HIV-1 Qual Whole Blood Test must be interpreted in conjunction with other clinical and laboratory findings.

The SAMBA II HIV-1 Qual Whole Blood Test is not intended to be used as a blood screening test for HIV-1 nor for viral load monitoring in HIV-1 infected individuals.

The SAMBA HIV-1 Qual Test has reduced sensitivity and reproducibility for Group O subtypes.

PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY[3]

The analytical sensitivity of the SAMBA II HIV-1 Qual Test was evaluated using a dilution series of the WHO 4th International HIV-1 standard (NIBSC 16/194) in negative whole blood. Panel members were prepared at 12000, 6000, 3000, 1500, 750 and 375 IU/ml or copies/ml and tested in 24 replicates using three lots of reagents in multiple SAMBA II instruments. The SAMBA II HIV-1 Qual Test showed 100% positivity in all replicates at or higher than 3000 IU/ml. Probit analysis of the data in Table 1a, using the WHO 4th HIV-1 International HIV-1 Standard, determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability, was 1909 IU/ml (95% CI: 1391-3008 IU)/ml).

Table 1a: Analytical sensitivity of the SAMBA HIV-1 Qual Test in whole blood spiked with WHO 4th International HIV-1 RNA Standard.

Concentration (IU/ml)	Number Tested	Number detected	Number of invalidated results	Percent detected
12000	24	22	2	**100%**
6000	24	22	2	**100%**
3000	24	23	1	**100%**
1500	24	16	4	**83%**
750	24	17	0	**50%**
375	24	11	1	**0%**

A study using another calibrator (in copies/ml), the HIV-1 VQA RNA Quantification Standard (Virology Quality Assurance Standard from NIH-AIDS Reagent Program), was also carried out to determine the LOD. Dilutions tested were 3333, 1000, 333, 100 and 33 copies/ml in whole blood. Using the statistical software SPSS v29, Probit analysis of the data in Table 1b using the HIV-1 VQA RNA Quantification Standard, determined that the concentration of HIV-1 RNA in whole blood detected with 95% probability, was 744 copies/ml (95% CI: 503-1329 copies/ml).

Table 1b: Analytical sensitivity of the SAMBA HIV-1 Qual Test in whole blood spiked with HIV-1 VQA RNA Quantification Standard.

Copies/ml	Replicates	Number detected	Invalidated test results	Percent detected
3333	24	24	0	100
1000	24	22	1	96
333	24	20	1	87
100	24	4	1	17
33	24	0	2	0

Seroconversion sensitivity

The sensitivity of the SAMBA II HIV-1 Qual Test was evaluated by testing sequential plasma specimens from 10 HIV-1 seroconversion panels diluted 1:5 with negative whole blood. In total 60 samples were tested on SAMBA I and SAMBA II of which 30 were positive on both systems[1,3]. All samples with viral load >156 copies/ml were positive by SAMBA I and SAMBA II. Of the 30 samples that tested negative on SAMBA I and SAMBA II four samples should have had detectable virus, according to the manufacturers data, PRB949-2 (130 copies/ml), PRB956-2 (142 copies/ml), PRB962-3 (152 copies/ml) and PRB968-5 (130 copies/ml) but these are below the limit of detection of the SAMBA HIV-1 Qual Test (Table 2).

Table 2: Seroconversion sensitivity

Panel member	Bleed date	Comparator NAT (cp/ml)*	SAMBA II HIV-1 Qual Whole Blood Test result
PRB949-1	0	BLD[a]	Negative
PRB949-2	6	100 [a]	Negative
PRB949-3	9	2,000 [a]	Positive
PRB949-4	18	120,000 [a]	Positive
PRB950-1	0	BLD[b]	Negative
PRB950-2	18	14,000 [b]	Positive
PRB950-3	21	100,000 [b]	Positive
PRB950-4	28	100,000 [b]	Positive
PRB956-1	0	BLD[d]	Negative
PRB956-2	40	142[d]	Negative
PRB956-3	42	1,440[d]	Positive
PRB956-4	47	32,000[d]	Positive
PRB956-5	50	60,000[d]	Positive
PRB962-1	0	<50[e]	Negative
PRB962-2	2	<50[e]	Negative
PRB962-3	7	152[f]	Negative
PRB962-4	9	1,540 [f]	Positive
PRB962-5	14	140,000 [f]	Positive
PRB962-6	17	2,400,000 [f]	Positive
PRB963-1	0	<50 [e]	Negative
PRB963-2	2	<50 [e]	Negative
PRB963-3	7	<50 [e]	Negative
PRB963-4	9	<50 [e]	Negative
PRB963-5	14	1,560 [f]	Positive
PRB963-6	17	19,200 [f]	Positive
PRB963-7	21	124,000 [f]	Positive
PRB964-1	0	<50[e]	Negative
PRB964-2	3	<50[e]	Negative

Panel member	Bleed date	Comparator NAT (cp/ml)*	SAMBA II HIV-1 Qual Whole Blood Test result
PRB964-3	10	<50[e]	Negative
PRB964-4	15	400[f]	Positive
PRB964-5	17	4,800[f]	Positive
PRB964-6	22	11,600[f]	Positive
PRB967-1	0	0[f]	Negative
PRB967-2	3	12[f]	Negative
PRB967-3	7	1,340[f]	Positive
PRB967-4	17	118,000[f]	Positive
PRB967-5	19	1,800,000[f]	Positive
PRB967-6	24	36,000[f]	Positive
PRB968-1	0	BLD[d]	Negative
PRB968-2	3	BLD[d]	Negative
PRB968-3	8	BLD[d]	Negative
PRB968-4	10	BLD[d]	Negative
PRB968-5	15	130[d]	Negative
PRB968-6	17	1,860[d]	Positive
PRB968-7	26	860,000[d]	Positive
PRB968-8	28	1,380,000[d]	Positive
PRB968-9	33	200,000[d]	Positive
PRB968-10	35	26,000[d]	Positive
PRB975-1	0	BLD[d]	Negative
PRB975-2	2	BLD[d]	Negative
PRB975-3	7	11[d]	Negative
PRB975-4	9	156[d]	Positive
PRB975-5	14	162,000[d]	Positive
PRB978-1	0	BLD[d]	Negative
PRB978-2	2	BLD[d]	Negative
PRB978-3	19	BLD[d]	Negative
PRB978-4	21	BLD[d]	Negative
PRB978-5	26	20[d]	Negative
PRB978-6	28	740[d]	Positive
PRB978-7	33	200,000[d]	Positive

*Final cp/ml: Calculated based on manufacturer's package insert after 1:5 dilution in whole blood
BLD = below limit of detection
[a] Roche PCR data provided by manufacturer
[b] Roche Amplicor HIV-1 Monitor data provided by manufacturer
[c] Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 data provided by manufacturer
[d] Abbott HIV RNA m2000 data provided by manufacturer
[e] Roche Ultra Sensitive HIV-1 RNA data provided by manufacturer
[f] Roche Standard HIV-1 RNA data provided by manufacturer

DETECTION OF HIV-1 SUBTYPES AND GROUPS[1]

The performance of the SAMBA II HIV-1 Qual test at detecting different HIV-1 subtypes and groups was evaluated by testing a minimum of 10 clinical specimens of common group M subtypes A (10), B (10), C (11), D (10) and circulating recombinant forms: CRF01_AE (12), F (10), CRF02_AG (12) and G (11). For the rarer Group M subtypes, two clinical specimens of subtype K, three of subtype J and 10 replicates of one sample for subtype H were tested. Fourteen Group O clinical specimens and 10 replicates of one Group N sample were also tested (Table 3). Each sample was diluted in negative whole blood to a concentration of 1200 copies/mL (3 X LOD). The Group O subtypes were tested at 1 200, 2 500, 8 000 and 25,000 copies/mL. Thirteen of the fourteen were detected between these concentrations with weak signals[1].

Table 3: Subtype detection of SAMBA Qual Test on SAMBA II

Subtype	Number tested	Number detected
A	10	10/10
B	10	10/10
C	11	11/11
D	10	10/10
AE	12	12/12
F	10	10/10
AG	12	12/12
G	11	11/11
H	1 (x10 replicates)	10/10
J	3	3/3
K	2	2/2
N	1 (x10 replicates)	10/10
O	14**	13/14**

** The samples tested positive between 1,200 and 25,000 copies/mL

ANALYTICAL SPECIFICITY

The analytical specificity of the SAMBA II HIV-1 Qual Test chemistry was evaluated using 503 seronegative whole blood samples in K_3EDTA 25 from Seracare and 478 from the German Red Cross. The result was negative for all 503 of the samples and the specificity of the SAMBA HIV-1 Qual assay was calculated to be 100% (503/503).

Related infections

The following retroviruses and other blood-borne infectious pathogens or sexually transmitted infections were evaluated for potential cross-reactivity with the SAMBA II HIV-1 Qual test: HIV-2 (5), HTLV-I (5), HTLV-II (4), Epstein Barr Virus (1), Hepatitis B (5) and Hepatitis C (5). Each was tested unspiked, diluted 1:3 with negative whole blood and spiked with 1800 IU/mL of the WHO HIV-1 RNA 3rd International standard. No cross reactivity nor interference was observed in unspiked or spiked samples respectively.

Specimen types

A panel of 25 HIV-1 negative blood donor samples obtained as matched plasma in K2EDTA, K3EDTA and sodium citrate were tested to determine the effect of anticoagulants on the results of the SAMBA II HIV-1 Qual assay. All of the whole blood samples had been certified as negative for HIV-1 RNA, Anti-HIV1/2, Anti-HCV, HCV RNA, non-reactive for HBsAg, and non-reactive for syphilis by the supplier.

Using 3 lots of reagents, the 25 sets of matched samples were each spiked with 1800 IU/mL of the WHO HIV-1 3rd International standard and tested along with unspiked samples. One hundred percent (100%) of the spiked samples were reactive, irrespective of the anticoagulant (K2EDTA, K3EDTA and sodium citrate). All unspiked samples were negative indicating that the above anticoagulants do not interfere with the SAMBA II HIV-1 Qual Test.

Interfering Substances

The SAMBA II HIV-1 Qual Test was evaluated with a panel of endogenous interfering substances which included Bilirubin, Triglycerides, Human DNA, ANA positive plasma, Lupus, Multiple Myloma, Multiple Sclerosis and Rheumatoid factor. These samples were tested spiked with 1800 IU/ml of WHO 3rd HIV-1 international standard and unspiked. No interference or cross reactivity was observed in any of these conditions.

In addition, the SAMBA II HIV-1 Qual Test with whole blood sample was also tested in the presence of antiviral drugs including Kivexa, Atripla, Kaletra, Combivir, Nevirapine, Ribavirin and Sequanivir. These were tested spiked with 1800 IU/ml of WHO 3rd International standard and unspiked. No interference or cross reactivity was observed in the presence of these drugs.

Cross Contamination

To further demonstrate the robustness of the assay, 10 runs of alternating positive spiked sample (50, 000 IU/ml) and negative sample were carried out using the same SAMBA II assay module. Results showed 100% agreement with the expected result in both negative and positive samples indicating no carry-over or cross-contamination occurred when running the assay.

Whole system failure rate

To assess the whole system failure rate, 100 replicates of negative whole blood were spiked with VQA HIV-1 quantification standard to contain 1,200 IU/ml (~3 x LOD) and were analyzed by a single operator over a period of 2 days using one lot of reagents of the SAMBA II HIV-1 Qual Whole Blood Test. All the 100 samples were reactive indicating no false-negative result was obtained from the 100 runs. There was no system failure observed during the 100 runs.

Reproducibility and Accuracy

Reproducibility testing for the SAMBA II HIV-1 Qual Whole Blood Test was assessed according to the Clinical and Laboratory Standards Institute (formerly NCCLS) Guideline (EP 12-A2, User Protocol for Evaluation of Qualitative Test Performance: Approved Guideline, 2nd edition). A 3-member panel was prepared using the 3rd WHO International HIV-1 RNA standard in normal human whole blood: 5,000 IU/ml (Medium Positive), and 0 IU/ml (Negative) and a HIV-1 positive clinical sample with <50 copies/ml (Low Positive) and were masked. Each operator tested a total of 40 samples using one lot of reagent each over 5 days. Results from the study indicate that there was 100% concordance between the expected result and the generated result using the SAMBA II HIV-1 Qual Whole Blood Test. In addition, the results demonstrate that the SAMBA II HIV-1 Qual Whole Blood Test showed good reproducibility as shown by 100% agreement between operators, between-run, between-lot, within-run, and between-day (Table 4).

Table 4. Summary of results from the SAMBA II HIV-1 Qual Whole Blood Test reproducibility study

Parameters	Negative (0 IU/ml)	Low Positive (HIV-positive <50cp/ml)	Medium positive (5,000 IU/ml)
Total number of replicates	40	40	40
Between-Operator	NEG (100% agreement)	POS (100% agreement)	POS (100% agreement)
Between-Day	NEG (100% agreement)	POS (100% agreement)	POS (100% agreement)
Between-Run	NEG (100% agreement)	POS (100% agreement)	POS (100% agreement)

CLINICAL PERFORMANCE

The SAMBA II HIV-1 Qual Whole Blood Test (P/N 4500-12) is a variant of the SAMBA I HIV-1 Qual Whole Blood Test (P/N 4200-12). Both tests have exactly the same chemistry and test conditions. Equivalency in performance between the two systems has been demonstrated in the data shown in the analytical sensitivity, specificity and subtype detection between the SAMBA I HIV-1 Qual Whole Blood Test (C08-0004-EN) and the SAMBA II HIV-1 Qual Whole Blood Test (see above under performance specifications).

The SAMBA I HIV-1 Qual Whole Blood Test has been evaluated in the field extensively in multiple countries (Uganda[4], Kenya[4], Malawi and Zimbabwe[4]) with a sensitivity of 100% (95% CI, 95.3-100) and specificity of 99.2% (95% CI, 95.6-99.9) in 202 adult samples and sensitivity of 98.6% (95% CI, 96.7-99.5) and specificity of 99.8% (95% CI, 99.1-100) in 942 infant samples.

The clinical performance of the SAMBA II HIV-1 Qual Whole Blood Test was evaluated using a subset of leftover frozen samples from the SAMBA I field evaluations in Uganda (36 adult and 83 infant samples) and 89 consecutive fresh whole blood samples from HIV-infected/exposed infants (≤24 months) on-site at Bwaila Hospital, Lilongwe, Malawi. The SAMBA II results were compared to CAP/CTM Qualitative Test using DBS Test (Uganda) or the Abbott Real-Time Qualitative Assay using DBS (Malawi) and to the SAMBA I results using fresh whole blood. In total 42 positive and 166 negative samples were detected. The sensitivity of SAMBA II was 100% (95% CI 91.6-100) and specificity was 100% (95% CI 97.8-100) in these 208 samples (Table 5).

Table 5: Summary of SAMBA II HIV-1 Qual Whole Blood Test performance in whole blood samples from Uganda and Malawi.

Country	Patients	Assay	Sample type	% sensitivity (95% CI)	% specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Uganda	Adults (n=36)	SAMBA I	Fresh WB	100 (77.9-100)	100 (88.2-100)	100 (77.9-100)	100 (88.2-100)
		SAMBA II	Frozen WB	100 (77.9-100)	100 (88.2-100)	100 (77.9-100)	100 (88.2-100)
	Infants (n=83)	SAMBA I	Fresh WB	100 (76.2-100)	100 (95.9-100)	100 (76.2-100)	100 (95.9-100)
		SAMBA II	Frozen WB	100 (76.2-100)	100 (95.9-100)	100 (76.2-100)	100 (95.9-100)
Malawi	Infants (n=89)	SAMBA I	Fresh WB	100 (82.4-100)	100 (94.8-100)	100 (82.4-100)	100 (94.8-100)
		SAMBA II	Fresh WB	100 (82.4-100)	100 (94.8-100)	100 (82.4-100)	100 (94.8-100)
TOTAL	**208**	**SAMBA I**		**100 (91.6-100)**	**100 (97.8-100)**	**100 (91.6-100)**	**100 (97.8-100)**
		SAMBA II		**100 (91.6-100)**	**100 (97.8-100)**	**100 (91.6-100)**	**100 (97.8-100)**

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REVISION HISTORY

Version no.	Brief description of changes	Date approved
12	Corrected typos on page 15 for Limit of Detection and Table 1b. Updated Technical Support contacts and added revision history. Updates made for clarity and completeness of document, thus risk is low.	30 May 23
13	Clarified warnings and precautions for venous and capillary whole blood sample collection. Included text citation for the references provided in the IFU. Removed any reference to EC Rep. Updates were made to improve clarity and correct an oversight, thus risk is low.	27 Jul 23

Technical Support

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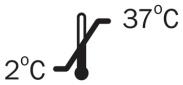
Symbol	Meaning
	**Catalogue**
	**Batch or Lot**
	**Store at 2-37ºC**
	***In Vitro* Diagnostic medical**
	**Use-by-date (YYYY-MM)**
	**Consult Instructions for Use**
	**Do not use if package is damaged**
	**Do not re-use**
	**Hazard warning**
	**Manufacturer:**
	**Keep dry**
	**Fragile, handle with care**
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