Diagnostics for the Real World

SAMBA III SCoV-2/Flu/RSV Test

(Simple nucleic acid AMplification Based Assay)

REF 9000-12

IVD

INTENDED USE

The SAMBA III SCoV-2/Flu/RSV Test is a qualitative, multiplex, nucleic acid amplification test (NAAT) based on reverse transcription polymerase chain reaction (RT-PCR) technology for the simultaneous detection and differentiation of RNA from SARS-CoV-2, Influenza A, Influenza B, and/or Respiratory Syncytial Virus (RSV) in nasopharyngeal, oropharyngeal, or combined nose and throat swab specimens collected in Universal Transport Medium (UTM), Viral Transport Medium (VTM), or SAMBA III Specimen Buffer.

The test is intended for use in individuals who present with signs and symptoms of respiratory tract infection, in conjunction with clinical and epidemiological information, to aid in the diagnosis of infection caused by the listed pathogens. The test is designed for use by trained healthcare professionals in a clinical laboratory setting or near patient environments, using the fully automated SAMBA III instrument system.

The detection of viral RNA indicates the presence of the virus but may not represent the causative agent for clinical symptoms. Negative results do not preclude infection with other pathogens and should not be used as the sole basis for patient management decisions. Positive results do not rule out co-infection with other pathogens.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The SAMBA III SCoV-2/Flu/RSV Test is a fully automated (RT-PCR)-based assay run on the SAMBA III instrument system consisting of the SAMBA III Assay Module and the SAMBA III Tablet Module control unit. The SAMBA III Hub is also available as an optional accessory for data management. All reagents are pre-loaded in sealed containers and ready to use without any requirement for preparatory activities prior to running the assay.

The SAMBA III SCoV-2/Flu/RSV Test requires the use of an Amplification tube and three (3) cartridges that hold reagents and materials for extraction of viral nucleic acids from the specimen, amplification of the nucleic acid target(s) and the detection of the amplification products. All the assay steps which take place sequentially within specific cartridges are driven by the mechanical movement of the different parts of the Assay Module. Filters are built-in to further prevent cross-contamination and ensure the safety of the user.

Sample extraction: The extraction phase of the assay involves the lysis of the viral and/or bacterial target(s) and the release of the nucleic acid into solution, which is then captured by a silica membrane column. The bound nucleic acid is washed and then eluted from the membrane.

Amplification and Detection: The SAMBA III SCoV-2/Flu/RSV Test target sequences undergo reverse transcription and real-time multiplexed polymerase chain reaction amplification and detection in the sealed SAMBA III amplification/detection tube. Detection is via an optical system equipped with filters which detect the different fluorescent tags specific for each target and read the Ct value, which is then converted into a result via a pre-determined algorithm in the Assay Module. The assay result is readable in the SAMBA III Tablet Module via a user interface, which can then print results using a Bluetooth printer. Although the assay is qualitative, the number of cycles may be accessed for additional information purposes only. The assay is provided with a built-in internal control (Bacteriophage MS2) to serve as a control throughout the assay. [1]. The internal control is present to check for correct assay function, from extraction to amplification and detection. The internal control is lyophilized in a sphere with other extraction reagents.

Results are for the detection of RNA of the following viruses: SARS-CoV-2, Influenza A, Influenza B, and RSV . These pathogens are generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of active infection.

Negative results do not preclude any of the targets and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

PREVENTION OF NUCLEIC ACID CONTAMINATION

Nucleic acid amplification-based assays are extremely sensitive and at risk of false positive results when contamination occurs. It is highly recommended that the user reads the care and maintenance instructions for the SAMBA instrument system and observes good laboratory practice during SAMBA testing and disposal of used test materials.

MATERIALS AND REAGENTS REQUIRED

SAMBA III SCoV-2/Flu/RSV Test Kit (List No. (9000-12)

NOTE: *All reaction tubes and cartridges required to test 12 samples using the SAMBA III SCoV-2/Flu/RSV Test Kit are packaged in a Kit Box. Inside the Kit Box are 2 compartments, each containing two (2) vacuum packs. The kit contains 12 cartridges each containing dry and liquid materials: S3 Quad Cartridge 1, S3 Quad Cartridge 2, S3 Quad Cartridge 3 and S3 Quad Cartridge 4. S3 Quad Cartridge 1&3 packed together, while S3 Quad Cartridge 2&4 are packed together in pouches. Twelve (12) tubes of SAMBA III Specimen Buffer and a bag of 12 sample collection tubes are also provided with the kit. Each Kit Box has enough cartridges and materials to test 12 samples. All cartridges/tubes are for single use only.*

9000A & 9000C **12 each** **S3 Quad Cartridge 1 & S3 Quad Cartridge 3**

Each S3 Quad Cartridge 1 consists of the amplification tube used for the assay. Each S3 Quad Cartridge 1 is for single-use only.

Each S3 Quad Cartridge 3 contains two lyophilised spheres consisting of a PCR master mix of reverse transcriptase, DNA polymerase, buffers, salts, synthetic oligonucleotides specific to all target nucleic acids/internal control, internal control material, and a filter column. Each S3 Quad Cartridge 3 is single-use only.

9000B & 9000D **12 each** **S3 Quad Cartridge 2 & S3 Quad Cartridge 4**

Each S3 Quad Cartridge 2 contains tubes with extraction buffers for nucleic acids from swab samples. The buffers contain a mixture of salts, ProClin 300, ProClin 950, and detergent. S3 Quad Cartridge 2 also contains the disposable adapter. Each S3 Quad Cartridge 2 is for single use only.

Each S3 Quad Cartridge 4 contains pipet tips (consumables) and is for single use only.

C19-0125 **12 each** **SAMBA III Specimen Buffer, 2 ml**

Each tube contains 2 ml of SAMBA III Specimen Buffer which includes a detergent that inactivates viruses and bacteria.

Other materials required and provided

C19-0130-12 S3 Sample Collection Tube
C20-0009-12 Transfer pipets, 453 µl

Other materials required but <u>not</u> provided

Flocked, Rayon or Dacron swabs with plastic shaft for sample collection.
Pipettor and tips capable of delivering 200µl (When using samples collected in UTM)

Equipment to be ordered separately

I19-0007-AM SAMBA III Assay Module **(required)**
I19-0007-TM SAMBA III Tablet Module **(required)**
I19-0007-HM SAMBA III Hub and Data Management system (optional)
SAMBA printer (optional)

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WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use Only

Safety Precautions

- *Handle all specimens as though capable of transmitting infectious agents. Note that the SAMBA III Specimen Buffer inactivates the target viruses and bacteria; hence, these samples can be handled at the point of care; however, samples collected in UTM/VTM will require a BSC II hood following BSL II requirements for preparation, as the highly infectious viruses will not be inactivated.*
- *Wear disposable powder-free gloves, laboratory 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.*
- *Read these Instructions for Use and the SAMBA III instrument system User Guides completely before using this product, and follow the instructions carefully.*
- *Do not use kit or its components after stated **kit expiry date**.*
- *Dispose of all contaminated materials, such as cartridges and tips, in accordance with local, state, 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.*
- *Clean and disinfect all work surfaces thoroughly with a nucleic acid cleaning agent. Freshly prepared 0.5% hypochlorite solution may also be used to clean surfaces and cavities of the SAMBA III Assay Module, followed by distilled water and 70% ethanol or isopropyl alcohol, then allowed to dry before use.*

S3 Quad Cartridge 2 and SAMBA III Specimen Buffer contain liquid buffers with components of known hazardous substances including ProClin and Tergitol. These components are classified per applicable European Community Directives as:

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

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

Hazard statements:

- *H317: May cause an allergic skin reaction*
- *H315: Causes skin irritation*
- *H318: Causes serious eye damage*

Precautionary Statements Prevention

- *P261: Avoid breathing dust/fume/gas/mist/vapours/ spray*
- *P264: Wash hands thoroughly after handling*
- *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

- *P302+P352: If on skin, wash with plenty of soap and water.*
- *P305+P351+P338+ P310: If in eyes, rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do; continue rinsing. Immediately call a poison center/ doctor.*
- *P333+P313: If skin irritation or rash occurs, get medical advice/attention.*
- *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.*
- *P501: Dispose of contents/container to an approved waste disposal plant*

Reagent storage and handling instructions

- *Store the kit at 2-30°C*
- *Keep all cartridges/tubes 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*

INSTRUMENT REQUIREMENTS

The SAMBA III SCoV-2/Flu/RSV Test is performed using the SAMBA III instrument system consisting of the SAMBA III Assay Module (P/N I19-0007-AM), SAMBA III Tablet Module *(*P/N I19-0007-TM) and SAMBA III Hub Module (P/N I19-0007-HM) (optional) is available as an optional external tool for data management (**NOTE:** The SAMBA III Hub Module is **not** required to run the assay). The SAMBA III 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. [2a, 2b].

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SPECIMEN COLLECTION AND HANDLING INSTRUCTIONS

1. *NOTES:*
    a. *As some of the viral targets (e.g. SARS CoV-2) are extremely infectious, collect and handle specimens with appropriate PPE prescribed by your institution.*
    b. *Samples collected in SAMBA III Specimen Buffer, which inactivates the viruses and bacteria, can be handled at the point-of-care; however, samples collected in UTM/VTM will require a BSC II hood following BSL II requirements for preparation of the sample as the viruses will not be inactivated.*
2. Collect specimens from combined nose and throat swabs and nasopharyngeal/oropharyngeal swabs according to the swab manufacturer's instructions.
3. Flocked, Rayon or Dacron swabs with plastic shafts are preferred for collecting respiratory samples. Proper specimen collection, storage, and transport are critical to the performance of this test (see Limitations of the test, page 16).
4. For swab specimens placed in UTM/VTM, proceed to Section A.1.a.

    (NOTE: Remel M4RT and *MWE Virocult collection media diluted at 1:2 in SAMBA III Specimen Buffer are suitable for use with the SAMBA III SCoV-2/Flu/RSV Test. Other transport media have not been validated with the SAMBA III SCoV-2/Flu/RSV Test, thus, their performance with the assay has not been assessed).*
5. For swab specimens placed in SAMBA III Specimen Buffer, proceed to Section A.1.b.
6. Storage temperature and time:
    a) Swab specimens may be held in SAMBA III Specimen Buffer for up to 24 hours at 30°C or up to 7 days at 2-8°C prior to testing. Freezing of samples should be avoided but for archiving purposes, may remain stable at -80°C for up to 12 months if freeze/thawing is avoided.
    b) For specimens collected in UTM/VTM, please refer to UTM/VTM manufacturer's guidelines for stability claims, which are summarized below:

    MWE Virocult: 96 hours stability at room temperature as per manufacturer package insert (Doc No. IFU-SIGMA-VIROCULT®-).

    Remel M4RT; 48 hours stability at room temperature as per manufacturer package insert (Doc No. IFUS12505)

    Copan UTM-RT; 96 hours stability at room temperature as per manufacturer package insert (Doc No. EIFU058R01-Date-2025.03_UTM)

    For optimum results, however, samples should be tested within the same day of collection. For refrigerated swab specimens, allow the sample to equilibrate to room temperature prior to testing.

7. For optimum results, samples should be tested within the same day of collection. Freezing/thawing of samples should be avoided but if needed for archiving purposes, samples frozen at -80°C for up to 9 months are acceptable for testing with SAMBA III SCoV-2/Flu/RSV Test.

ASSAY PROCEDURE

READ these instructions for use and the SAMBA III instrument system User Guides carefully before performing the test. Don appropriate PPE according to your institution's health and safety requirements.

The SAMBA III SCoV-2/Flu/RSV Test is intended to be used only with the SAMBA III instrument system.

- *Do not use kits beyond the kit expiration date.*
- *Components within a kit box are intended to be used together.*
- *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 III Assay Module.*
- *Records of the test results must be kept as per the established protocols of your organisation or institution.*

1. **Switch on the SAMBA III Assay Module.** The internal light will illuminate, and the status indicator strip on the front of the module will show white, and the module number will appear. The AM is now ready for use.
2. **Switch on the SAMBA III Tablet Module.** On the TM, select the AM number that you wish to use to run the assay (the Module Number from the front of the AM will be displayed on the TM).

Additional notes:

Set up the SAMBA III instrument system before starting the assay:

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

A. SAMPLE PREPARATION

1. **NOTE: Follow your routine procedure for handling swabs including appropriate PPE and BSL requirements.**
    a. **For swabs in Universal / Virus Transport medium (NOTE: Perform the following steps in a BSC II hood and follow BSL II requirements as UTM samples have not been inactivated).**
        i. Mix well and open the lid of the SAMBA III Specimen Buffer (Code C19-0125).
        ii. Using a pipettor and pipette tips capable of dispensing 200µl, transfer 200 µl of the buffer into the Sample Collection Tube (red cap) provided.
        iii. Transfer 200 µl of the sample in transport medium into the same Sample Collection Tube (red cap) provided.

iv. Mix by pipetting up and down gently three times. Cap the tube and leave the sample for 10 minutes inside the tube prior to use to inactivate the sample.

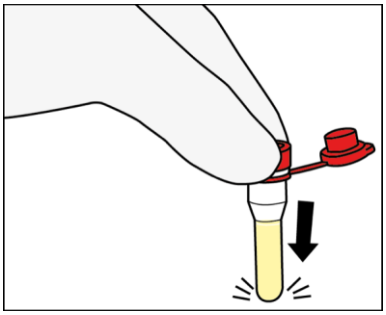
b. **Sample collection using SAMBA III Specimen Buffer (Code C19-0125)**

i. Insert the swab into the SAMBA III Specimen Buffer (Code C19-0125) tube and break at the marked point on the shaft. Discard the shaft as potentially infectious material.

ii. Soak the swab for 10 minutes in the SAMBA III Specimen Buffer.

iii. Mix the sample by shaking it side to side.

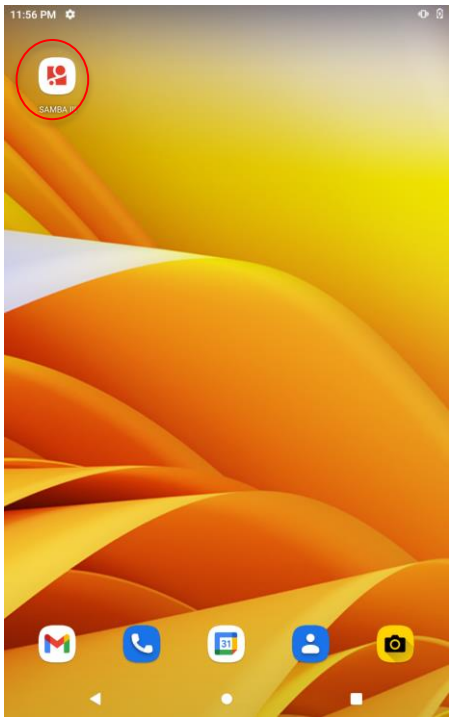
iv. Using the transfer pipet provided with the kit, transfer 400 µl of the inactivated sample into the Sample Collection Tube and cap securely.



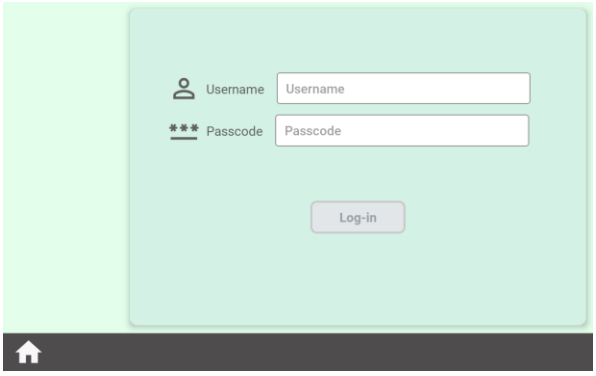
2. Proceed to run the assay (Section B).

B. RUNNING THE ASSAY USING THE SAMBA III INSTRUMENT SYSTEM

1. Switch on the Tablet and unlock using the PIN or password set for device (Refer to User Guide for SAMBA III Tablet Module for detailed instructions- Doc no C19-0123). Locate the SAMBA III App icon on the Home screen. Tap the SAMBA III 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.


Username
Username
Passcode
Passcode
Log-in

All results

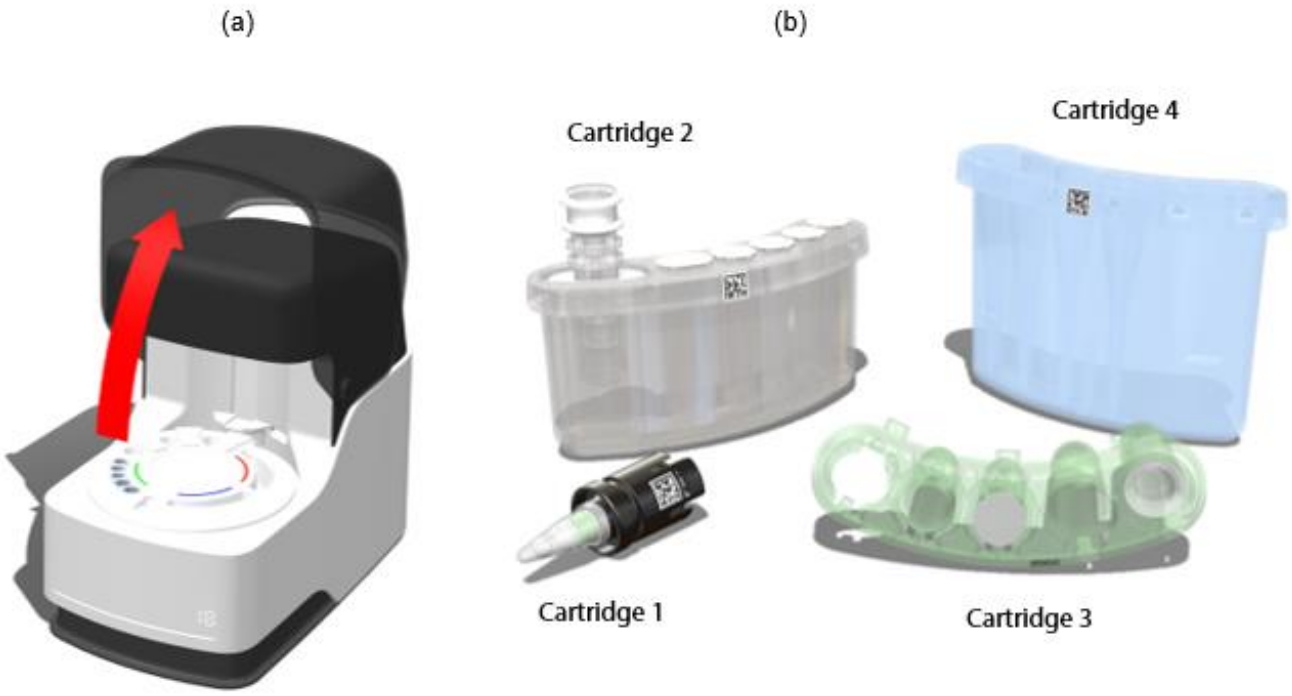
C. Set up the SAMBA III Assay Module.

1. Set up the SAMBA III SCoV-2/Flu/RSV Test Cartridges on the SAMBA III Assay Module.
2. Open the kit box and remove a set of 2 bags (combined S3 Quad Cartridge 1 & S3 Quad Cartridge 3 and combined S3 Quad Cartridge 2 & S3 Quad Cartridge 4).
3. Confirm the contents:

   1 each S3 Quad Cartridge 1 & S3 Quad Cartridge 3 (List no. 9000A and List no. 9000C)

   1 each S3 Quad Cartridge 2 & S3 Quad Cartridge 4 (List no. 9000B and List no. 9000D)

4. Using the Tablet Module, scan the 2D barcode on the S3 Quad Cartridge 2 and 4 bag to identify the assay type. Carefully remove the cartridges from the bags. Visually inspect each cartridge to ensure foil seals are not perforated and cartridges and tubes are securely clipped.
5. Open the AM lid to its full extent (a) (it will stay open unaided) and inspect the cartridges (b).



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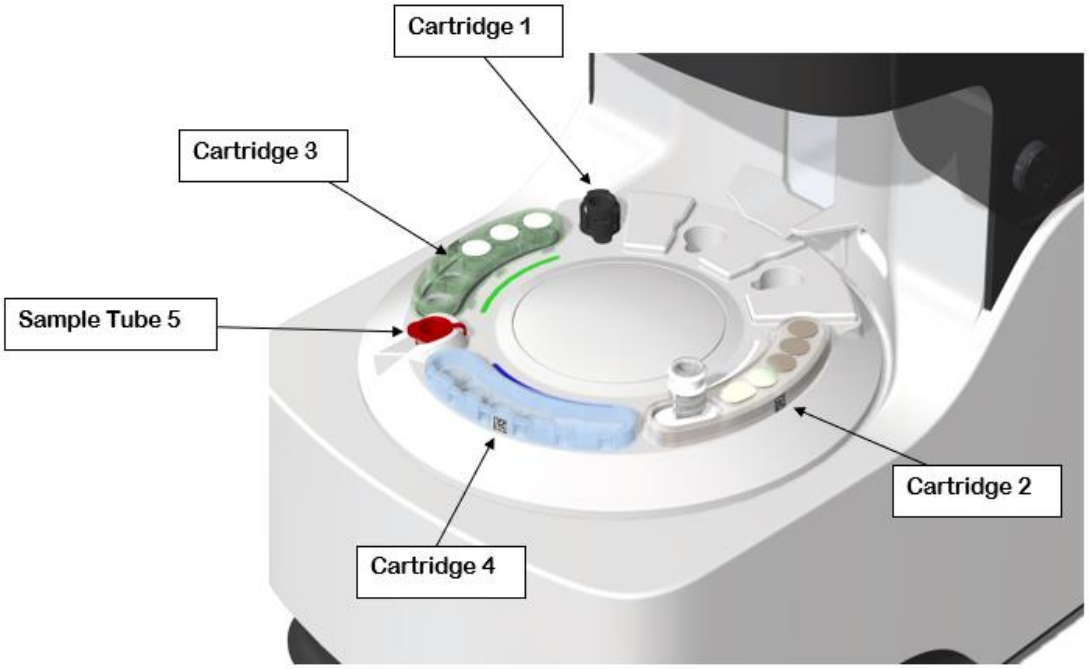
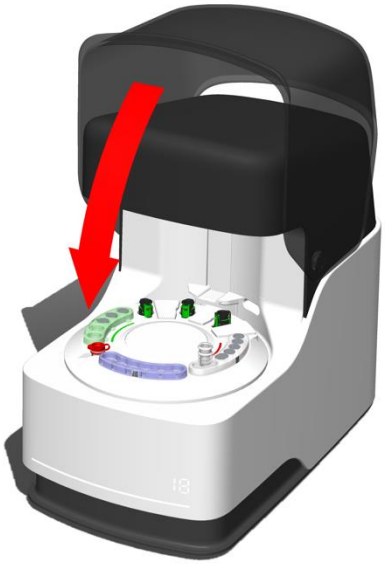
6. Load cartridges into the Assay Module following the diagram below:
   - Remove S3 Quad Cartridge 1 and S3 Quad Cartridge 3 from the pouch.
   - Insert S3 Quad Cartridge 1 into position 1.
   - Insert S3 Quad Cartridge 3 into position 3.
   - Remove S3 Quad Cartridge 2 and S3 Quad Cartridge 4 from the pouch.
   - Insert S3 Quad Cartridge 2 into position 2.
   - Insert S3 Quad Cartridge 4 into position 4.
   - Scan the 2D barcode on the Sample Collection Tube containing the sample and load into in the sample holder (see 5 in the figure on page 12):

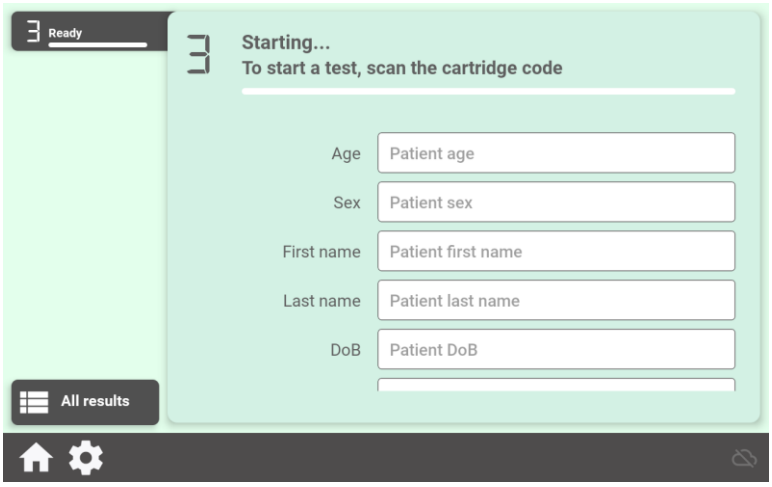


7. Ensure that all cartridges are fully inserted and snugly fit before proceeding. The turret can be rotated if needed to make it easier to load the cartridges. (See the User Guide for Assay Module, Doc No. C19-0122, for details on how to load the cartridges and samples).

8. Close the lid.



9. Fill out the mandatory fields on the tablet module.



10. The start button will now be available on the Tablet Module. The AM is now ready to begin testing. Press the Start button to proceed.

> ⚠ DO NOT OPEN THE LID at any time during a test. This will stop the test and any results will be invalid.
>
> Please refer to the SAMBA III Tablet Module User Guide
>
> (Doc No C19-0123) for information on how to run a test.

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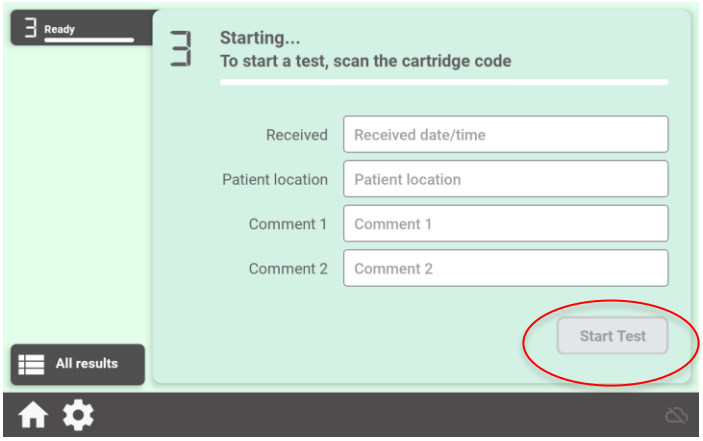
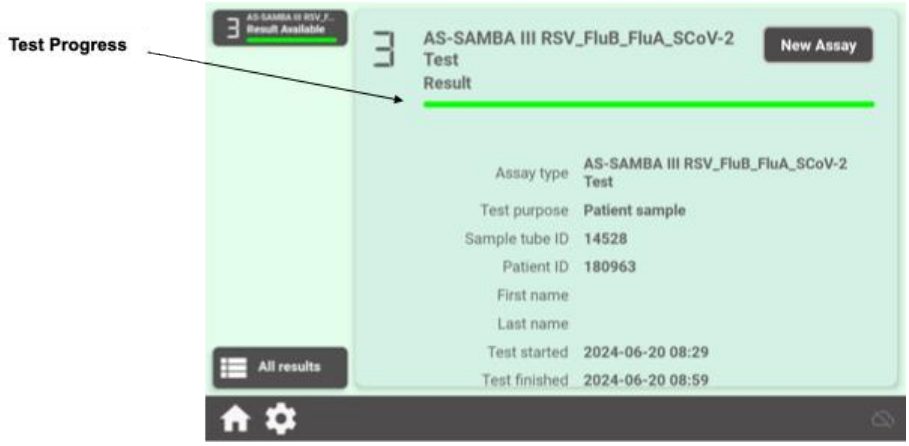


Once the test has started, a Test Progress bar will appear on the screen. The stripe on the front of the Assay Module will change from white to green. The details for the test running will appear on the Test Progress screen on the Tablet Module as shown below. The entire assay will run for approximately 35 minutes.



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

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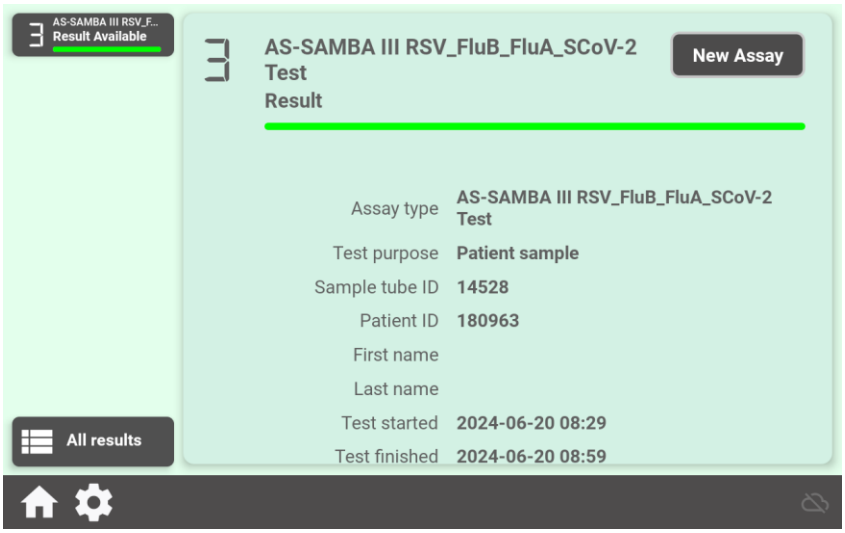

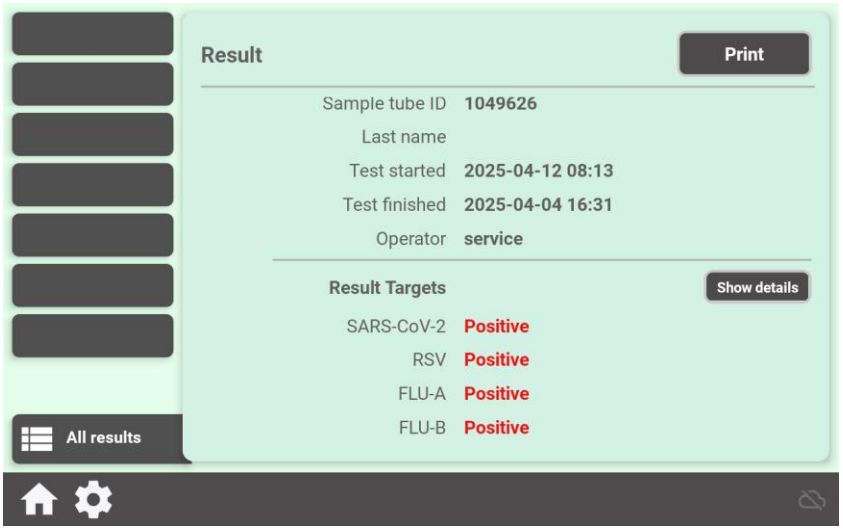

12. Once the assay is completed, the AM will come to a stop, and the status indicator strip on the front of the machine will appear white. The DMS on the TM will also indicate that the test has finished. The test cartridges may now be removed. **Note:** Used cartridges and sample tube are biohazardous and should be disposed of according to the established procedures for such materials.
13. Once all the cartridges and sample tube have been removed, the SAMBA III AM may either be switched off or prepared for another test.

Note: *A record of the test result must be kept with patient records in accordance with the established protocols of your organisation or institution. To achieve this, the Test Result screen has 2 buttons- PRINT and START NEW ASSAY .If required or as an option, send result to DMS according to the Tablet Module User Guide. Refer to Tablet Module User Guide (Doc No. C19-0123) for details on connecting to Bluetooth printer to print results and uploading test results to the SAMBA III Hub Module. To upload the result to a LIMS, refer to the User Guide for SAMBA III Hub/DM Module (C19-0124).*

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INTERPRETATION OF RESULTS

A. Reading of results

At the end of the assay, one of the following eleven (11) results appears on the SAMBA III Tablet Module.

Note: In case of multiple targets, more than one of the targets may be detected.

Tablet Module read-out	Result
SARS-CoV-2 Positive	SARS-CoV-2 was detected.
SARS-CoV-2 Negative	SARS-CoV-2 was **not** detected.
RSV Positive	RSV was detected.
RSV Negative	RSV was **not** detected.
Flu B Positive	Influenza B was detected.
Flu B Negative	Influenza B was **not** detected.
Flu A Positive	Influenza A was detected.
Flu A Negative	Influenza A was **not** detected.
Invalid	Repeat testing*
Halted	Repeat testing*
No result	Repeat testing*

**Test must to be repeated using fresh sample and cartridges. For a repeat invalid result, which may be due to inhibitory substances intrinsic to the sample itself or extremely high viral load, the sample can be tested using an alternate platform or by carrying out further dilution (e.g. 1:10 using SAMBA III Specimen Buffer or as per the institution's standard operating procedure).*

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QUALITY CONTROL

The Internal Control signal should always serve to verify, in the absence of a test result, that each specimen was processed correctly and that no inhibitors of amplification were present. In samples with high organism load, the internal control may not appear. An external positive control may be run with the SAMBA III SCoV-2/Flu/RSV Test after routine validation per laboratory's standard operating procedure.

Limitations of the Test

For *in vitro* diagnostic use only.

- The SAMBA III SCoV-2/Flu/RSV Test has been validated for qualitative detection of SARS-CoV-2, Influenza A, Influenza B and/or RSV RNA in respiratory specimens such as combined nasal + throat and nasopharyngeal/oropharyngeal swab specimens.. Test reagents and procedures should be run in conjunction with the SAMBA III instrument system.
- Clinical performance of the SAMBA III SCoV-2/Flu/RSV Test has only been established in combined nasal and throat clinical swab specimens.
- Flocked, rayon, or dacron swabs are preferred for collecting respiratory samples. As per current recommendations by Public Health England, urethral, pernasal, or mini-tip swabs may also be alternatives if standard-sized swabs are not available. 'Charcoal' swabs (such as swabs transported in a charcoal-containing medium) are not suitable. Swabs with polystyrene shafts should be used, as swabs with wooden shafts may contain PCR inhibitors and may also be a safety issue.
- Improper sampling techniques may result in insufficient organism load, potentially leading to a reduced assay sensitivity.
- The SAMBA III SCoV-2/Flu/RSV Test requires personnel trained on the SAMBA III instrument system to run the instrument and the assay procedures.
- The SAMBA III SCoV-2/Flu/RSV Test procedures and instrument system have been designed to reduce the risk of contamination with the 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.
- The following nasal sprays have been found to interfere with the SAMBA III SCoV-2/Flu/RSV Test: Benconase, Pirinase and Benacort.. Therefore, it is highly recommended to ask the patient to refrain from using nasal sprays prior to sample collection.
- The assay is provided with a built-in internal control to serve as a control throughout the assay, however, on rare occasions, the following limitations may occur:

    - Partial Workflow Monitoring- The internal control is not an endogenous substance, so it does not monitor the quality of the sample, such as sample degradation during transport or improper storage.
    - Interpretation of Control Failure- A failed internal control (invalid result) may result from poor sample quality or presence of inhibitory substances in the specimen analyzed or pipetting errors while the test is running. Additional investigation may be required to determine the root cause, and repeat testing is required.
    - Reagent Competition- The internal control has been titrated to allow low concentration (LOD) of target to be detected. LOD testing has been performed with a set concentration of internal control, and testing has confirmed that there is no competition observed at our limit of detection for each assay target, but there is a chance it may compete with very low-level target sequences (beyond our claim) for amplification reagents especially in multiplex assays.

- As with any other *in vitro* diagnostic test, the detection of SARS-CoV-2/ Influenza A/ Influenza B/ RSV 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 III SCoV-2/Flu/RSV Test must be interpreted in conjunction with other clinical and laboratory findings.

PERFORMANCE EVALUATION

The performance characteristics of the SAMBA III SCoV-2/Flu/RSV Test were determined using various reference materials, including prepared panels and clinical specimens.

- NIBSC Working Standard Winter Respiratory Panel for the following individual targets: Influenza A H1N1 (Code 20/180), Influenza A H3N2 (Code 20/182), Influenza B (Code 20/184), human Respiratory Syncytial Virus (RSV) A (Code 20/186) and human Respiratory Syncytial Virus (RSV) B (Code 20/188)
- WHO First International Standard for SARS CoV-2 RNA (NIBSC Code 20/146) [5]
- Clinical specimens
- Microorganisms from the AR Bank, American Type Culture Collection[6], BEI Resources[7], EVAg [8] and ZeptoMetrix [9] (See Table 2b for details)

Analytical sensitivity (Limit of detection)

The Limit of detection (LoD) of the SAMBA III SCoV-2/Flu/RSV Test on combined nasal + throat swab specimens I two buffers (SAMBA III Specimen Buffer and UTM) using specimens spiked with different concentrations of SARS CoV-2, Influenza A (H1N1 or H3N2) or RSV was estimated by Probit analysis.

A. SAMBA III Specimen Buffer

SARS-CoV-2

Using the First WHO International Standard for SARS CoV-2 RNA (NIBSC Code 20/146, five (5) concentrations (350, 250, 150, 100 and 50 IU/ml) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer were tested in 24 replicates per concentration. The LoD was confirmed at **174 IU/ml** (95% CI:115-194) (Table 1A)

Influenza A H1N1

For Influenza A H1N1, the LoD was determined by testing five (5) concentrations (100, 75, 50, 35 and 25 U/mL) diluted in pooled negative combined nose and throat swab specimens collected in SAMBA III Specimen Buffer in 24 replicates per concentration. The final LOD was confirmed at **41 U/ml** (95% CI: NA-44) (Table 1A).

Influenza A H3N2

For Influenza A H3N2, the LoD was determined by testing five (5) concentrations (125, 100, 75, 50 and 25 U/mL) diluted in pooled negative combined nose and throat swab specimens collected in SAMBA III Specimen Buffer in 24 replicates per concentration. The final LOD was confirmed at **74 U/ml** (95% CI:46-83) (Table 1A).

Influenza B

For Influenza B, the LoD was determined by testing five (5) concentrations (350, 250, 150, 100 and 50 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in 24 replicates per concentration. The final LoD was confirmed at **153 U/mL** (95% CI 89-176) (Table 1A).

Respiratory Syncytial Virus (RSV) A
For Respiratory Syncytial Virus (RSV) A, the LoD was determined by testing six (6) concentrations (300, 250, 200, 150, 100 and 50 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in at least 24 replicates per concentration. The final LoD was estimated at **138 U/mL** (95% CI: 40-228) (Table 1A).

Respiratory Syncytial Virus (RSV) B
For Respiratory Syncytial Virus (RSV) B, the LoD was determined by testing five (5) concentrations (250, 200, 150, 100 and 50 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in at least 24 replicates per concentration. The final LoD was estimated at **144 U/mL** (95% CI: 105-161) (Table 1A).

B. UTM

SARS-CoV-2
Using the First WHO International Standard for SARS CoV-2 RNA (NIBSC Code 20/146, five (5) concentrations (700, 500, 300, 200 and 100 IU/ml) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer were tested in 24 replicates per concentration. The final LoD was confirmed at **245 IU/ml** (95% CI: NA-268) (Table 1B).

Influenza A H1N1
For Influenza A H1N1, the LoD was determined by testing five (5) concentrations (200, 150, 100, 75 and 50 U/mL) diluted in pooled negative combined nose and throat swab specimens collected in SAMBA III Specimen Buffer in 24 replicates per concentration. The final LOD was confirmed at **96 U/ml** (95% CI: 64 – 109) (Table 1B).

Influenza A H3N2
For Influenza A H3N2, the LoD was determined by testing by testing five (5) concentrations (250, 200, 150, 100 and 75 U/mL) diluted in pooled negative combined nose and throat swab specimens collected in SAMBA III Specimen Buffer in 24 replicates per concentration. The final LOD was confirmed at **141 U/ml** (95%CI: 87 – 165) (Table 1B).

Influenza B
For Influenza B, the LoD was determined by testing five (5) concentrations (500, 400, 300, 200 and 100 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in 24 replicates per concentration. The final LoD was confirmed at **297 U/ml** (95% CI: 135 – 381) (Table 1B).

Respiratory Syncytial Virus (RSV) A
For Respiratory Syncytial Virus (RSV) A, the LoD was determined by testing five (5) concentrations (400, 350, 300, 250 and 200 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in at least 24 replicates per concentration. The final LoD was estimated at **294 U/ml (**95% CI: 222-322**)** (Table 1B).

Respiratory Syncytial Virus (RSV) B
For Respiratory Syncytial Virus (RSV) B, the LoD was determined by testing five (5) concentrations (400, 350, 300, 250 and 200 U/mL) diluted in pooled negative combined nose and throat swab specimens in SAMBA III Specimen buffer in at least 24 replicates per concentration. The final LoD was estimated at **300 U/ml** (95% CI: 208-343) (Table 1B).

Table 1A: LOD of the SAMBA III SCoV-2/Flu/RSV Test in SAMBA III Specimen Buffer

Microorganism	Virus source and strain	LoD	95% CI
SARS-CoV-2	First WHO International Standard for SARS-CoV-2 RNA (NIBSC code 20/146)	174 IU/ml	115-194
Influenza A (H1N1)	NIBSC Working Standard Winter Respiratory Panel for Influenza A H1N1 (20-180)	41 U/ml	NA-44
Influenza A (H3N2)	NIBSC Working Standard Winter Respiratory Panel for Influenza A H3N2 (20-182)	74 U/ml	46 – 83
Influenza B	NIBSC Working Standard Winter Respiratory Panel for Influenza B (20-184)	153 U/ml	89 - 176
RSV A	NIBSC Working Standard Winter Respiratory Panel for RSV-A (20-186)	138 U/ml	40-228
RSV B	NIBSC Working Standard Winter Respiratory Panel for RSV-B (20-188)	144 U/ml	105-161

Table 1B: LOD of the SAMBA III SCoV-2/Flu/RSV Test in Universal Transport Medium

Virus	Virus source and strain	LoD	95% CI:
SARS-CoV-2	First WHO International Standard for SARS-CoV-2 RNA (NIBSC code 20/146)	245 IU/ml	NA -268
Influenza A (H1N1)	NIBSC Working Standard Winter Respiratory Panel for Influenza A H1N1 (20-180)	96 U/ml	64 – 109
Influenza A (H3N2)	NIBSC Working Standard Winter Respiratory Panel for Influenza A H3N2 (20-182)	141 U/ml	87-165
Influenza B	NIBSC Working Standard Winter Respiratory Panel for Influenza B (20-184)	297 U/ml	135 – 381
RSV A	NIBSC Working Standard Winter Respiratory Panel for RSV-A (20-186)	294 U/ml	222-322
RSV B	NIBSC Working Standard Winter Respiratory Panel for RSV-B (20-188)	300 U/ml	208-343

Analytical reactivity (Inclusivity)

To assess the analytical reactivity (inclusivity) of the SAMBA III SCoV-2/Flu/RSV Test (P/N 9000), a panel of different strains of Influenza A, Influenza B, RSV and SARS-CoV-2 was spiked into pooled negative nose/throat swab specimens at low concentration(~3X LoD).. Results are summarized in Table 2.

Analytical specificity (Cross-reactivity/exclusivity)

Low concentration of a panel of SARS CoV-2 variants, Influenza A, Influenza B, RSV A and RSV B were analysed in 3 lots of pre-masterlots (one replicate/lot) of the SAMBA III SCoV-2/Flu/RSV Test for reactivity by wet testing. All the strains of SARS CoV-2, Flu A, Flu B, RSV A and RSV B were detected (Table 2).

Table 2: Inclusivity panel of SARS CoV-2, Influenza A, Influenza B, RSV A and RSV B strains

Strain	Final concentration tested	Kit batch Number	S/N of Assay Module SIII AM-S1-	Tablet result interpretation
Heat-inactivated SARS-CoV-2 variant B.1.1.7	450cp/ml	Pre-ML 1	0070	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 2	0005	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 3	0035	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
Heat-inactivated SARS-CoV-2 variant B.1.351 (Beta)	450cp/ml	Pre-ML 1	0003	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 2	0057	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 3	0046	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
Heat-inactivated SARS-CoV-2, Omicron variant	450cp/ml	Pre-ML 1	0012	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 2	0056	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 3	0006	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
Heat-inactivated SARS-CoV-2, Delta variant	450cp/ml	Pre-ML 1	0073	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 2	0040	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
		Pre-ML 3	0028	**SARS CoV-2 detected**, RSV not detected, FluA not detected, FluB not detected
Influenza A virus (H3N2)	135 $TCID_{50}$/ml	Pre-ML 1	0023	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0010	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0024	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Genomic RNA from Influenza A virus, A/duck/Ukraine/ 1963 (H3N8) -- Influenza A virus	0.31ng/100µl	Pre-ML 1	0070	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0068	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0045	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected

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Strain	Final concentration tested	Kit batch Number	S/N of Assay Module SIII AM-S1-	Tablet result interpretation
Influenza A virus A/Hong Kong/H090-756-V1(0)/2009 (H3N2)	134 $TCID_{50}$/ml	Pre-ML 1	0048	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0051	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0060	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Influenza A virus (H1N1)	135 $TCID_{50}$/ml	Pre-ML 1	0067	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0001	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0054	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Genomic RNA from Influenza A Virus, A/duck/Germany/1215/1973 (H2N3)	0.31ng/100µl	Pre-ML 1	0053	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0016	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0068	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Influenza A Virus, A/Puerto Rico/8-MC/1934 (H1N1)	135 $CEID_{50}$/ml	Pre-ML 1	0044	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0036	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0032	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Influenza A virus A/Brisbane/59/2007 (H1N1)	134 $CEID_{50}$/ml	Pre-ML 1	0072	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0052	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0047	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Influenza A Virus, A/turkey/Wisconsin/1/1966 (H9N2) (uncloned)	134 $CEID_{50}$/ml	Pre-ML 1	0037	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 2	0026	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
		Pre-ML 3	0039	SARS CoV-2 not detected, RSV not detected, **FluA detected**, FluB not detected
Influenza B virus B/Taiwan/2/1962	280 $CEID_{50}$/ml	Pre-ML 1	0004	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 2	0021	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 3	0014	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
Influenza B virus B/Malaysia/2506/2004	280 $CEID_{50}$/ml	Pre-ML 1	0070	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 2	0005	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 3	0035	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
Influenza B Virus --	**280 $CEID_{50}$/ml**	Pre-ML 1	0003	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**

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Strain	Final concentration tested	Kit batch Number	S/N of Assay Module SIII AM-S1-	Tablet result interpretation
B/Sydney/507/2006 (Yamagata Lineage)		Pre-ML 2	0057	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 3	0046	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
Influenza B virus -- Kilbourne F59: B/Massachusetts/1/1971 (HA) x B/Lee/1940 (NA)	**280 $CEID_{50}$/ml**	Pre-ML 1	0012	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 2	0056	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 3	0006	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
Influenza B virus	**280 $CEID_{50}$/ml**	Pre-ML 1	0073	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 2	0040	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
		Pre-ML 3	0028	SARS CoV-2 not detected, RSV not detected, FluA not detected, **FluB detected**
Human respiratory syncytial virus A1998/3-2 (Strain A)	**28 $TCID_{50}$/ml**	Pre-ML 1	0023	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 2	0010	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 3	0024	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
Human respiratory syncytial virus (Strain B)	**28 $CEID_{50}$/ml**	Pre-ML 1	0070	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 2	0068	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 3	0045	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
Human respiratory syncytial virus (Strain B)	**28 $TCID_{50}$/ml**	Pre-ML 1	0048	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 2	0051	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected
		Pre-ML 3	0060	SARS CoV-2 not detected**, RSV detected**, FluA not detected, FluB not detected

***In silico* analysis**

The NCBI BLASTing tool was used to blast primers and probes against Adenovirus (e.g. Adenovirus 2 and 5), Human Metapneumovirus (hMPV), Parainfluenza virus 1-4, Influenza A, Influenza B, Enterovirus (e.g. B80), Respiratory syncytial virus (RSV), Rhinovirus, *Chlamydia pneumoniae, Haemophilus influenzae, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumoniae, Streptococcus pyogenes, Bordetella pertussis, Mycoplasma pneumoniae, Pneumocystis jirovecii* (PJP), *Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus salivarius,* SARS-CoV-1, SARS-CoV-2, Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKNI63, SARS-CoV HKU339849, MERS-coronavirus, *Corynebacterium diphtheriae, Legionella pneumophila, Bacillus anthracis (Anthrax), Moraxella catarrhalis, Neisseria elongata, Neisseria meningitidis, Morbillivirus* (Measles) and *Varicella zoster* (chicken pox). Degenerate nucleotides were automatically accounted for as a mismatch and query cover was used as a proxy for non-specific hybridisation and hereafter referred to as 'percentage of homology'. When a percentage of homology

was found to be equal to or above 80%, the alignments were further studied, and the ratio of matching nucleotides was indicated.

Results from *in silico* analysis (Table 3a to Table 3d) showed that the SARS-CoV-2 and RSV primers and probes used in the SAMBA III SCoV-2/Flu/RSV Test showed less than 80% homology with all the microorganisms tested; however, the FluA and FluB primers or probe showed >80% homology with some microorganisms, e.g.

- The FluB_F2 forward primer showed a homology of 80% with *Pseudomonas aeruginosa* however, the FluB_R2 reverse primer did not show homology in that same region.
- The FluB_P2 probe showed a homology of 95% (which is above the threshold of 80%) with *Streptococcus epidermidis*; however, the FluB_F2 and R2 primers did not show homology in that same region.
- The FluB_R1 reverse primer showed a homology of 83.3%, with *Neisseria elongata;* however, the FluB_F1 forward primer did not show homology in that same region
- The FluA_P1 probe has shown a homology of 93.5%, with *Pseudomonas aeruginosa***;** however, the FluB_F2 and R2 primers did not show homology in that same region.

In all the above instances, either the reverse (or forward) primers or probes did not show homology in the same regions; hence, amplification could not take place. Consequently, the absence of an amplified product should prevent detection of off-target signals. Wet testing with these organisms also gave negative results for Flu A and Flu B (and the rest of the targets), indicating non-cross-reactivity with the SAMBA III SCoV-2/Flu/RSV test.

Table 3. Percent homology of SARS-CoV-2 primers and probes used in the SAMBA III SCoV-2/Flu/RSV test against the high priority organisms likely in the circulating area.

High priority organisms likely in the circulating area	Primer ORF1ab F	Primer ORF1ab R	Primer ORF1ab P	Primer ORF8 F	Primer ORF8 R	Primer ORF8 P
Adenovirus (e.g. C1 Ad. 71)	<80%	<80%	<80%	<80%	<80%	<80%
Human Metapneumovirus (hMPV)	<80%	<80%	<80%	<80%	<80%	<80%
Parainfluenza virus 1-4	<80%	<80%	<80%	<80%	<80%	<80%
Influenza A	<80%	<80%	<80%	<80%	<80%	<80%
Influenza B	<80%	<80%	<80%	<80%	<80%	<80%
Enterovirus (e.g. EV68)	<80%	<80%	<80%	<80%	<80%	<80%
Respiratory syncytial virus	<80%	<80%	<80%	<80%	<80%	<80%
Rhinovirus	<80%	<80%	<80%	<80%	<80%	<80%
Chlamydia pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Haemophilus influenzae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Mycobacterium tuberculosis	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pyogenes	<80%	<80%	<80%	<80%	<80%	<80%
Bordetella pertussis	<80%	<80%	<80%	<80%	<80%	<80%

High priority organisms likely in the circulating area	Primer ORF1ab F	Primer ORF1ab R	Primer ORF1ab P	Primer ORF8 F	Primer ORF8 R	Primer ORF8 P
Mycoplasma pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Pneumocystis jirovecii (PJP)	<80%	<80%	<80%	<80%	<80%	<80%
Candida albicans	<80%	<80%	<80%	<80%	<80%	<80%
Pseudomonas aeruginosa	<80%	<80%	<80%	<80%	<80%	<80%
Staphylococcus epidermidis	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus salivarius	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV-1	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV-2						
Human coronavirus 229E	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus OC43	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus HKNI63	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV HKU339849	<80%	<80%	<80%	<80%	<80%	<80%
MERS-coronavirus	<80%	<80%	<80%	<80%	<80%	<80%
Corynebacterium diphtheriae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Bacillus anthracis (*Anthrax*)	<80%	<80%	<80%	<80%	<80%	<80%
Moraxella catarrhalis	<80%	<80%	<80%	<80%	<80%	<80%
Neisseria elongata	<80%	<80%	<80%	<80%	<80%	<80%
Neisseria meningitidis	<80%	<80%	<80%	<80%	<80%	<80%
Morbillivirus (Measles)	<80%	<80%	<80%	<80%	<80%	<80%
Varicella zoster (chicken pox)	<80%	<80%	<80%	<80%	<80%	<80%

Table 3b. Percent homology of Influenza A primers and probes used in the SAMBA III SARS-CoV-2/Flu/RSV test against the high priority organisms likely in the circulating area.

High priority organisms likely in the circulating area	Primer FluA_F1	Primer FluA_R 1	Primer FluA_P1	Primer FluA_F2	Primer FluA_R2	Primer FluA_P2
Adenovirus (e.g. C1 Ad. 71)	<80%	<80%	<80%	<80%	<80%	<80%
Human Metapneumovirus (hMPV)	<80%	<80%	<80%	<80%	<80%	<80%
Parainfluenza virus 1-4	<80%	<80%	<80%	<80%	<80%	<80%
Influenza A						

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High priority organisms likely in the circulating area	Primer FluA_F1	Primer FluA_R 1	Primer FluA_P1	Primer FluA_F2	Primer FluA_R2	Primer FluA_P2
Influenza B	<80%	<80%	<80%	<80%	<80%	<80%
Enterovirus (e.g. EV68)	<80%	<80%	<80%	<80%	<80%	<80%
Respiratory syncytial virus	<80%	<80%	<80%	<80%	<80%	<80%
Rhinovirus	<80%	<80%	<80%	<80%	<80%	<80%
Chlamydia pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Haemophilus influenzae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Mycobacterium tuberculosis	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pyogenes	<80%	<80%	<80%	<80%	<80%	<80%
Bordetella pertussis	<80%	<80%	<80%	<80%	<80%	<80%
Mycoplasma pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Pneumocystis jirovecii (PJP)	<80%	<80%	<80%	<80%	<80%	<80%
Candida albicans	<80%	<80%	<80%	<80%	<80%	<80%
Pseudomonas aeruginosa	<80%	<80%	93.5% (15/16)	<80%	<80%	<80%
Staphylococcus epidermidis	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus salivarius	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV-1	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV-2	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus 229E	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus OC43	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus HKNI63	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV HKU339849	<80%	<80%	<80%	<80%	<80%	<80%
MERS-coronavirus	<80%	<80%	<80%	<80%	<80%	<80%
Corynebacterium diphtheriae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella non-pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Bacillus anthracis (*Anthrax*)	<80%	<80%	<80%	<80%	<80%	<80%
Moraxella catarrhalis	<80%	<80%	<80%	<80%	<80%	<80%
Neisseria elongata	<80%	<80%	<80%	<80%	<80%	<80%

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High priority organisms likely in the circulating area	Primer FluA_F1	Primer FluA_R 1	Primer FluA_P1	Primer FluA_F2	Primer FluA_R2	Primer FluA_P2
Neisseria meningitidis	<80%	<80%	<80%	<80%	<80%	<80%
Morbillivirus (Measles)	<80%	<80%	<80%	<80%	<80%	<80%
Varicella zoster (chicken pox)	<80%	<80%	<80%	<80%	<80%	<80%

Table 3c. Percent homology of Influenza B primers and probes used in the SAMBA III SCoV-2/Flu/RSV test against the high priority organisms likely in the circulating area.

High priority organisms likely in the circulating area	Primer FluB_F1	Primer FluB_R 1	Primer FluB_P1	Primer FluB_F2	Primer FluB_R2	Primer FluB_P2
Adenovirus (e.g. C1 Ad. 71)	<80%	<80%	<80%	<80%	<80%	<80%
Human Metapneumovirus (hMPV)	<80%	<80%	<80%	<80%	<80%	<80%
Parainfluenza virus 1-4	<80%	<80%	<80%	<80%	<80%	<80%
Influenza A	<80%	<80%	<80%	<80%	<80%	<80%
Influenza B						
Enterovirus (e.g. EV68)	<80%	<80%	<80%	<80%	<80%	<80%
Respiratory syncytial virus	<80%	<80%	<80%	<80%	<80%	<80%
Rhinovirus	<80%	<80%	<80%	<80%	<80%	<80%
Chlamydia pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Haemophilus influenzae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Mycobacterium tuberculosis	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Streptococcus pyogenes	<80%	<80%	<80%	<80%	<80%	<80%
Bordetella pertussis	<80%	<80%	<80%	<80%	<80%	<80%
Mycoplasma pneumoniae	<80%	<80%	<80%	<80%	<80%	<80%
Pneumocystis jirovecii (PJP)	<80%	<80%	<80%	<80%	<80%	<80%
Candida albicans	<80%	<80%	<80%	<80%	<80%	<80%
Pseudomonas aeruginosa	<80%	<80%	<80%	80% (16/20)	<80%	<80%
Staphylococcus epidermidis	<80%	<80%	<80%	<80%	<80%	95% (19/20)
Streptococcus salivarius	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV-1	<80%	<80%	<80%	<80%	<80%	<80%

DRW Diagnostics for the Real World

High priority organisms likely in the circulating area	Primer FluB_F1	Primer FluB_R 1	Primer FluB_P1	Primer FluB_F2	Primer FluB_R2	Primer FluB_P2
SARS-CoV-2	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus 229E	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus OC43	<80%	<80%	<80%	<80%	<80%	<80%
Human coronavirus HKNI63	<80%	<80%	<80%	<80%	<80%	<80%
SARS-CoV HKU339849	<80%	<80%	<80%	<80%	<80%	<80%
MERS-coronavirus	<80%	<80%	<80%	<80%	<80%	<80%
Corynebacterium diphtheriae	<80%	<80%	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%	<80%	<80%
Bacillus anthracis (*Anthrax*)	<80%	<80%	<80%	<80%	<80%	<80%
Moraxella catarrhalis	<80%	<80%	<80%	<80%	<80%	<80%
Neisseria elongata	<80%	83.3% (15/18)	<80%	<80%	<80%	<80%
Neisseria meningitidis	<80%	<80%	<80%	<80%	<80%	<80%
Morbillivirus (Measles)	<80%	<80%	<80%	<80%	<80%	<80%
Varicella zoster (chicken pox)	<80%	<80%	<80%	<80%	<80%	<80%

Table 3d. Percent homology of RSV primers and probes used in the SAMBA III SCoV-2/Flu/RSV test against the high priority organisms likely in the circulating area.

High priority organisms likely in the circulating area	Primer RSV_F	Primer RSV_R	Primer RSV_P1	Primer RSV_P2
Adenovirus (e.g. C1 Ad. 71)	<80%	<80%	<80%	<80%
Human Metapneumovirus (hMPV)	<80%	<80%	<80%	<80%
Parainfluenza virus 1-4	<80%	<80%	<80%	<80%
Influenza A	<80%	<80%	<80%	<80%
Influenza B	<80%	<80%	<80%	<80%
Enterovirus (e.g. EV68)	<80%	<80%	<80%	<80%
Respiratory syncytial virus				
Rhinovirus	<80%	<80%	<80%	<80%
Chlamydia pneumoniae	<80%	<80%	<80%	<80%
Haemophilus influenzae	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%
Mycobacterium tuberculosis	<80%	<80%	<80%	<80%
Streptococcus pneumoniae	<80%	<80%	<80%	<80%
Streptococcus pyogenes	<80%	<80%	<80%	<80%

DRW Diagnostics for the Real World

High priority organisms likely in the circulating area	Primer RSV_F	Primer RSV_R	Primer RSV_P1	Primer RSV_P2
Bordetella pertussis	<80%	<80%	<80%	<80%
Mycoplasma pneumoniae	<80%	<80%	<80%	<80%
Pneumocystis jirovecii (PJP)	<80%	<80%	<80%	<80%
Candida albicans	<80%	<80%	<80%	<80%
Pseudomonas aeruginosa	<80%	<80%	<80%	<80%
Staphylococcus epidermidis	<80%	<80%	<80%	<80%
Streptococcus salivarius	<80%	<80%	<80%	<80%
SARS-CoV-1	<80%	<80%	<80%	<80%
SARS-CoV-2	<80%	<80%	<80%	<80%
Human coronavirus 229E	<80%	<80%	<80%	<80%
Human coronavirus OC43	<80%	<80%	<80%	<80%
Human coronavirus HKNI63	<80%	<80%	<80%	<80%
SARS-CoV HKU339849	<80%	<80%	<80%	<80%
MERS-coronavirus	<80%	<80%	<80%	<80%
Corynebacterium diphtheriae	<80%	<80%	<80%	<80%
Legionella pneumophila	<80%	<80%	<80%	<80%
Bacillus anthracis (*Anthrax*)	<80%	<80%	<80%	<80%
Moraxella catarrhalis	<80%	<80%	<80%	<80%
Neisseria elongata	<80%	<80%	<80%	<80%
Neisseria meningitidis	<80%	<80%	<80%	<80%
Morbillivirus (Measles)	<80%	<80%	<80%	<80%
Varicella zoster (chicken pox)	<80%	<80%	<80%	<80%

Wet testing

Wet testing with available representative strains of microorganisms, including those that showed >80% homology with *in silico* analysis, at 10^6 cells/ml for bacteria/yeast and 10^2-10^5 pfu or $TCID_{50}$/ml for viruses (Table 4) in SAMBA III Specimen Buffer showed that SARS-CoV-2, Influenza A, Influenza B and/or RSV at low levels were detected by SAMBA III SCoV-2/Flu/RSV Test in spiked samples. The unspiked samples gave negative results indicating that the assay is specific and confirmed the results from *in silico* analysis.

Table 4. Specific strains used in wet testing for cross-reactivity.

Microorganism	Source	Accession number	Final concentration tested
Bacteria			
Acinetobacter calcoaceticus	ATCC	23055	10^5/ml
Arcanobacterium haemolyticum	ATCC	9345	10^5/ml
Burkholderia orbicola (formerly B. cepacia)	ATCC	17762	10^5/ml
Chlamydia trachomatis	ZeptoMetrix	801775	10^5/ml
Corynebacterium diphtheriae	ZeptoMetrix	801882	10^5/ml
Bordetella avium	ATCC	35086	10^5/ml
Bordetella bronchiseptica	ATCC	10580	10^5/ml
Bordetella hinzii	ATCC	51783	10^5/ml
Bordetella holmesii	ATCC	700052	10^5/ml
Chlamydophila pneumoniae TW-183	*ATCC*	*VR-2282*	10^5/ml
Corynebacterium striatum	ATCC	BAA-1293	10^5/ml
Enterobacter cloacae	AR Bank	#0038	10^5/ml
Escherichia coli	AR Bank	#0538	10^5/ml
Fusobacterium necrophorum subsp *necrophorum*	ATCC	27852	10^5/ml
Haemophilus influenzae	ATCC	33391	10^5/ml
Haemophilus influenzae	ATCC	49766	10^5/ml
Klebsiella pneumoniae	AR Bank	#0039	10^5/ml
Lactobacillus acidophilus	ZeptoMetrix	801540	10^5/ml
Lactobacillus plantarum	ZeptoMetrix	804436	10^5/ml
Legionella (Fluoribacter) bozemanae	ATCC	33217	10^5/ml
Legionella (Fluoribacter) dumoffii	ATCC	33279	10^5/ml
Legionella longbeachae	ZeptoMetrix	801577	10^5/ml
Legionella (Tatlockia) micdadei	ZeptoMetrix	801576	10^6/ml
Legionella pneumophila	ZeptoMetrix	804007	10^6/ml
Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130	ATCC	BAA-1198D-5	0.7 ug/100 µl
Moraxella catarrhalis	ATCC	8176	10^5/ml
Mycoplasma orale	ATCC	19524	10^5/ml
Mycoplasma hominis	ZeptoMetrix	804011	10^5/ml
Mycoplasma pulmonis	BEI	NR-3858	10^4/ml
Mycobacterium tuberculosis, HN878, gamma-irradiated whole cells	BEI	NR-49100	10^5/ml

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Microorganism	Source	Accession number	Final concentration tested
Neisseria elongata	ZeptoMetrix	801510	10^5/ml
Neisseria gonorrhoeae	ATCC	19424	10^5/ml
Neisseria meningitidis	ATCC	13090	10^5/ml
Proteus mirabilis	ATCC	12453	10^5/ml
Pseudomonas aeruginosa	ATCC	27853	10^5/ml
Serratia marcescens	AR Bank	#0091	10^5/ml
Staphylococcus aureus subsp *aureus*	ATCC	10832	10^5/ml
Staphylococcus epidermidis	ATCC	29887	10^5/ml
Streptococcus agalactiae	ATCC	13813	10^5/ml
Streptococcus dysgalactiae subsp *dysgalactiae*	ATCC	43078	10^5/ml
Streptococcus pneumoniae	ATCC	BAA-341	10^5/ml
Streptococcus pyogenes	ATCC	19615	10^5/ml
Streptococcus salivarius subsp *salivarius*	ATCC	13419	10^5/ml
Ureaplasma urealyticum	ATCC	27618	10^5/ml
Aspergillus flavus	ZeptoMetrix	801598	10^5/ml
Aspergillus fumigatus	ZeptoMetrix	801716	10^5/ml
Candida albicans	ATCC	10231	10^5/ml
Cryptococcus neoformans	ATCC	MYA-4564	10^5/ml
Pneumocystis jirovecii (carinii)	ATCC	PRA-159	10^5/ml
Adenovirus 21 - AV1645	BEI	NR-56500	10^5/ml
Adenovirus Type 31 Culture Fluid (1 mL)	ZeptoMetrix	0810073CF	10^4/ml
Adenovirus Type 40 Culture species F Strain Dugan Fluid (1 mL)	ZeptoMetrix	0810084CF	10^3/ml
Coxsackievirus A10	ATCC	VR-168	10^5/ml
Coxsackievirus A21	ATCC	VR-850	10^5/ml
Human Coxsackievirus B1, Conn-5	BEI	NR-58650	10^5/ml
Cytomegalovirus (CMV)	ZeptoMetrix	0810003CF	10^2/ml
Cytomegalovirus C9209	BEI	ARP-2082	10^5/ml
	ABI Adv Biotech	HFS/CMV C9209-1	
Enterovirus 71 - Tainan/4643/1998	BEI	NR-471	10^5/ml
Epstein-Barr Virus (EBV)	ZeptoMetrix	0810008CF	10^5/ml
Human Echovirus 30 Strain Bastianni	ATCC	VR-1660	10^5/ml
Herpes simplex Virus 1 (HSV1)	ATCC	AV-VR-1778	10^5/ml
Human herpes virus 1 (*Herpes simplex* virus 1 strain H5)	ATCC	VR-260	10^5/ml
Human Herpes Virus 6 (HHV6) Type 6B	ZeptoMetrix	0810072CF	10^5/ml
Herpes simplex virus Type 2 Culture fluid	ZeptoMetrix	0810217CF	10^3/ml
Influenza A Virus - Influenza A Virus, A/Puerto Rico/8-MC/1934 (H1N1)	BEI	NR-29022	10^6/ml
Influenza A virus - A/Hong Kong/H090-756-V1(0)/2009 (H3N2)	BEI	NR-44344	10^5/ml

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Microorganism	Source	Accession number	Final concentration tested
Influenza B virus - B/Taiwan/2/1962	BEI	NR-3181	10^4/ml
Influenza B Virus - B/Sydney/507/2006 (Yamagata Lineage)	BEI	NR-36526	10^4/ml
Measles Virus -MVs/Maryland.USA/18.18 [B3]	BEI	NR-52251	10^4/ml
Measles Virus	ZeptoMetrix	0810025CF	10^4/ml
Human Metapneumovirus TN/ 83-1211	BEI	NR-22227	10^4/ml
Mumps Virus	ZeptoMetrix	0810079CF	10^5/ml
Human Parainfluenza Virus 1, HPIV1/FRA/29221106/2009	BEI	NR-48680	10^4/ml
Human Parainfluenza virus 2 - Greer	BEI	NR-3229	10^4/ml
Human Parainfluenza virus Type 4 - M-25	BEI	NR-3237	10^2/ml
Human Parechovirus (HPeV)	ZeptoMetrix	0810147CF	10^5/ml
Human Respiratory Syncytial Virus - A1998/3-2	BEI	NR-28529	10^5/ml
Human respiratory syncytial virus A2	ATCC	VR-1540	10^2/ml
HCoV-229E	EVAg	011N-03868	10^4/ml
HCoV-NI63	EVAg	011N-03868	10^4/ml
HCoV-OC43	EVAg	011N-03868	10^4/ml
HCoV-SARS-CoV	EVAg	011N-03868	10^4/ml
HCoV-MERS-CoV	EVAg	011N-03868	10^4/ml

ATCC: American Type Culture Collection, 10801 University Blvd, Manassas, VA 20110, USA
BEI: 10801 University Blvd, Manassas, VA 20110 USA
EVAg: UVE-UMR190 Emerging Viral Diseases Medical Faculty, 27 Boulevard Jean Moulin - 13385 Marseille cedex 5 - France
AR Bank: Antimicrobial Resistance Isolate Bank (AR Isolate Bank), 1600 Clifton Road, Mail Stop: H17-3, Atlanta, GA 30329-4027 USA
ZeptoMetrix: 14957 Collection Center Drive, Chicago, IL, 60693-0149 USA

Competitive interference

Competitive interference on the SAMBA III SCoV-2/Flu/RSV Test caused by co-infections was evaluated by testing low concentrations (3X LoD) each of SARS-CoV-2, Flu A H1N1, Flu B, RSV-A and RSV-B in the presence of the other target strains at a higher concentration. The concentrations were 450 IU/ml for SARS-CoV-2, 150 U/ml for Flu A H1N1, 225 U/ml for FluA H3N2, 450 U/ml for Flu B, 450 U/ml for RSV-A or 450 U/ml for RSV-B.

Results showed that FluA H1N1, FluA H3N2, FluB, RSV A and RSV B at high concentrations (100,000 U/ml), did not inhibit the detection of SARS CoV-2 when present at low concentration. Similarly, the other targets were also detected in the presence of a high background of SARS-CoV-2 (Table 5).

Table 5. Summary of results (No. of positives/Total no. tested) from competitive inhibition study

Test virus at 3x LOD	Interferant virus (100,000 U/ml)	No. pos/Total no. tested
SARS-CoV-2 (450 IU/ml)	Flu A H1N1	3/3
	Flu A H3N2	3/3
	Flu B	3/3
	RSV A	3/3
	RSV B	3/3
Flu A H1N1 (150 U/ml)	Flu A H3N2	3/3
	Flu B	3/3
	RSV A	3/3
	RSV B	3/3
	SARS-CoV-2	3/3
Flu A H3N2 (225 U/ml)	Flu A H1N1	3/3
	Flu B	3/3
	RSV A	3/3
	RSV B	3/3
	SARS-CoV-2	3/3
Flu B (450 U/ml)	Flu A H1N1	3/3
	Flu A H3N2	3/3
	RSV A	3/3
	RSV B	3/3
	SARS-CoV-2	3/3
RSV A (450 U/ml)	Flu A H1N1	3/3
	Flu A H3N2	3/3
	FluB	3/3
	RSV B	3/3
	SARS-CoV-2	3/3
RSV B (450 U/ml)	Flu A H1N1	3/3
	Flu A H3N2	3/3
	FluB	3/3
	RSV A	3/3

DRW Diagnostics for the Real World

Test virus at 3x LOD	Interferant virus (100,000 U/ml)	No. pos/Total no. tested
	SARS-CoV-2	3/3
RSV B (450 U/ml)	Flu A H1N1	3/3
	Flu A H2N3	3/3
	FluB	3/3
	RSV A	3/3
	SARS-CoV-2	3/3

Potential interfering substances

The effect of potential interfering substances was evaluated on the following panel of elevated levels of endogenous substances (whole blood and human genomic DNA and medication/pain relievers used by symptomatic individuals such as nasal spray, nasal gel, lozenges, homeopathic allergy relief medicine antiviral medication). Additionally, nose and throat swab specimens were taken from individuals after ingesting common foodstuff, beverages and alcohols .With the exception of Benconase, Pirinase and Benacort, which interfered with the SAMBA test at 5% but not at 1%, no interference was observed with the rest of the substances/drugs/antiviral tested in both spiked and unspiked samples (Table 6). Additionally, none of the endogenous substances (human blood and human genomic DNA, common foodstuff, beverages or alcohol) inhibited or interfered with the SAMBA III SCoV-2/Flu/RSV Test.

Table 6: Results from assessing effect of potential interferants on the SAMBA III SCoV-2/Flu/RSV Test.

Name of substance	Active Ingredient(s)	Concentration tested	Inhibition (yes/no)
Control	None	NA	No
Beconase	0.05% Beclomethasone dipropionate	5%	Yes
		1%	No
Pirinase	0.05% Fluticasone propionate	5%	Yes
		1%	No
Benacort	Budesonide 64mg/spray	5%	Yes
		1%	No
Benylin chesty cough	Guaifenesin 50mg/5ml	5%	No
		1%	
RelonChem Allergy Relief	Loratadine 5mg/ml	5%	No
		1%	
Nurofen for children	Ibuprofen 100mg/5ml	5%	No
		1%	
Benylin Night cough	Diphenhydramine 7mg/ml & Levomenthol 0.55mg/ml	5%	No
		1%	
Sudafed Decongestant	Pseudoephedrine hydrochloride 30mg/5ml	5%	No
		1%	
Ventolin	Salbutamol sulfate 2mg/5ml	5%	No
		1%	
Calpol 6 plus	Paracetamol 250mg/5ml	5%	No
		1%	
Sterimar	Sea water isotonic solution	5%	No
		1%	
Snuffle babe	Sodium Chloride 0.9%, Benzalkonium Chloride	5%	No
		1%	

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Tamiflu	Oseltamivir 6mg/ml	5%	No
		1%	
Human Whole Blood	N/A	5%	No
		1%	
Human Genomic DNA	108μg/ml	20 µg/ml	No
		2 µg/ml	

Specimen stability in SAMBA III Specimen Buffer

The SAMBA III Specimen Buffer has been shown to be effective against all enveloped viruses as defined in EN 14476:2013 + A2:2019 Annex A when tested after 1-minute exposure. This, therefore, includes all coronaviruses and SARS-CoV-2 and the rest of the targets (Influenza A and B and Respiratory Syncytial virus). This indicates that the specimens collected in SAMBA III Specimen Buffer from symptomatic individuals may be handled in a Class II cabinet.

2-8°C storage: Results indicate that the SAMBA III Specimen Buffer maintained sample stability for SARS-CoV-2, Influenza, and RSV for up to 7 days when stored at 2-8°C. All replicates remained positive throughout the study, indicating consistent stability over the 7-day period.

30°C storage: Results demonstrate that the SAMBA III Specimen Buffer kept SARS-CoV-2, Influenza and RSV stable for up to 24 hours when stored at 30°C. However, beyond 24 hours, one replicate showed a negative result for SARS-CoV-2, Influenza B and RSV at the 48-hour mark, suggesting that sample integrity may have been compromised. Based on these findings, it is recommended that samples stored up to 30°C should be processed as soon as possible, or ensuring that they are tested within 24 hours to maintain stability.

Sample collection media compatibility

Three commonly used specimen transport media were tested for compatibility with the SAMBA III SCoV-2/Flu/RSV Test: Remel M4RT, MWE Virocult collection media and Copan UTM-RT. All three media were found suitable for use with the SAMBA III SCoV-2/Flu/RSV Test when diluted at 1:2 in SAMBA III Specimen Buffer.

Reproducibility

The reproducibility of the SAMBA III SCoV-2/Flu/RSV Test was assessed on three lots of reagents using low-level positive (3x LOD), medium-level positive (10x LOD) in pooled nasal + throat swab specimens spiked with SARS-CoV-2, Flu A H1N1, Flu B, RSV-A and negative swab sample. The reproducibility assessment was performed in SAMBA III buffer and Universal Transport Media (UTM). Each run included replicates of 8 by three operators. The results below demonstrated that the assay was reproducible across three lots and three operators (Table 7).

Table 7. Reproducibility testing on 3 lots of SAMBA III SCoV-2/Flu/RSV Test by three operators

Panel	Specimen Buffer Type	Concentration	Operator 1 (no. positive/ no. tested)	Operator 2 (no. positive/ no. tested)	Operator 3 (no. positive/ no. tested)	Total (no. positive/ no. tested)
SARS-CoV-2 low positive (3x LOD)	SAMBA III Specimen Buffer	450 IU/ml	8/8	8/8	8/8	24/24
SARS-CoV-2 medium positive (10x LOD)		1500 IU/ml	8/8	8/8	8/8	24/24
Flu A H1N1 low positive (3x LOD)		150 U/ml	8/8	8/8	8/8	24/24
Flu A H1N1 medium positive (10x LOD)		500 U/ml	8/8	8/8	8/8	24/24
Flu B low positive (3x LOD)		450 U/ml	8/8	8/8	8/8	24/24
Flu B medium positive (10x LOD)		1500 U/ml	8/8	8/8	8/8	24/24
RSV A low positive (3x LOD)		450 U/ml	8/8	8/8	8/8	24/24
RSV A medium positive (10x LOD)		1500 U/ml	8/8	8/8	8/8	24/24
Negative		NA	0/8	0/8	0/8	0/24
SARS-CoV-2 low positive (3x LOD)	UTM (Remel M4RT)	450 IU/ml	8/8	8/8	8/8	24/24
SARS-CoV-2 medium positive (10x LOD)		1500 IU/ml	8/8	8/8	8/8	24/24
Flu A H1N1 low positive (3x LOD)		150 U/ml	8/8	8/8	8/8	24/24
Flu A H1N1 medium positive (10x LOD)		500 U/ml	8/8	8/8	8/8	24/24
Flu B low positive (3x LOD)		450 U/ml	8/8	8/8	8/8	24/24
Flu B medium positive (10x LOD)		1500 U/ml	8/8	8/8	8/8	24/24

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Panel	Specimen Buffer Type	Concentration	Operator 1 (no. positive/ no. tested)	Operator 2 (no. positive/ no. tested)	Operator 3 (no. positive/ no. tested)	Total (no. positive/ no. tested)
RSV A low positive (3x LOD)		450 U/ml	8/8	8/8	8/8	24/24
RSV A medium positive (10x LOD)		1500 U/ml	8/8	8/8	8/8	24/24
Negative		NA	0/8	0/8	0/8	0/24

Whole system failure rate

To assess the whole system failure rate of the SAMBA III SCoV-2/Flu/RSV Test [10], 100 replicates of negative nasal + throat swab specimens spiked with 2X LoD of all the four targets (SARS CoV-1, Flu A, Flu B and RSV) and 25 replicates of unspiked samples were tested by a single operator using a single lot of reagents and four (4) instruments. All the samples gave the expected results, thus, zero failure rate was recorded.

Carryover study

Potential carryover cross-contamination was evaluated and assessed by alternately running samples with high viral load (100,000 IU/mL of SARS CoV-2 or 100,000 U/mL of Influenza A H1N1, Influenza A H3N2, RSV A or RSV B and negative samples (0 IU/mL or U/mL) in five consecutive runs, each run across a single SAMBA III Assay Module and a single SAMBA III Tablet Module. This was repeated across five SAMBA III Assay Modules and five SAMBA III Tablet Modules. No carryover contamination was recorded as all negative samples gave a negative result, and all spiked samples gave a positive result.

Performance evaluation in clinical specimens

In this clinical evaluation study, the TaqPath COVID-19 Combo kit was used as the comparator test for SARS-CoV-2. The TaqPath COVID-19, FluA/B, RSV Combo kit was used for discrepant analysis for SARS-CoV-2, and also as the comparator test for FluA, FluB and RSV. For testing of discrepant results, the SAMBA III, TaqPath COVID-19 Combo and TaqPath COVID-19, FluA/B, RSV Combo kit were repeat-tested simultaneously, and the final disposition was based on the repeat test result. The sensitivity, specificity and 95% CI were then calculated using the Medcalc diagnostic test calculator: https://www.medcalc.org/calc/diagnostic_test.php.

Clinical samples

A. Symptomatic vs asymptomatic

Below is the distribution of specimens from symptomatic and asymptomatic individuals:

UTM (469)

- 118 symptomatic SCoV-2 positive (51 Remel M4RT + 67 MWE Virocult collection medium
- 25 asymptomatic SCoV-2 positive (16 Remel M4RT + 9 MWE Sigma Virocult collection medium)
- 39 symptomatic Flu A positive
- 13 symptomatic Flu B positive
- 57 symptomatic RSV positive
- 3 symptomatic negative (3 SCoV-2-positive by TaqPath COVID-19 Combo Kit) (MWE Virocult collection medium)
- 214 asymptomatic negative (MWE Sigma Virocult collection medium)

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SAMBA Specimen Buffer (349)

- 100 symptomatic SCoV-2 positive
- 29 symptomatic Flu A positive
- 8 symptomatic Flu B positive
- 16 symptomatic RSV positive
- 196 asymptomatic negative

B. Distribution of Ct values (determined by TaqPath assays) for positive clinical samples

Table 8 summarises the number of positive samples per Ct category included in the clinical performance evaluation following the requirements of the UK's Coronavirus Test Device Approvals (CTDA).

Table 8 – Distribution of Ct values (determined by TaqPath assays) for positive clinical samples

Ct	UTM samples				SAMBA Specimen Buffer samples			
	SCoV-2	Flu A	Flu B	RSV	SCoV-2	Flu A	Flu B	RSV
<25	51	39	7	29	38	29	3	15
25-30	31	0	4	22	31	0	3	1
>30	64	0	2	6	31	0	2	0
Total	**146**	**39**	**13**	**57**	**100**	**29**	**8**	**16**

C. Results from testing with SAMBA III and TaqPath

Samples collected in UTM

SARS-CoV-2

In total, 146 SARS-CoV-2 positive combined nose/throat samples collected in UTM from both symptomatic and asymptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, 143 were positive by SAMBA III, and 3 were negative. The three negative results gave mean Ct values of 34.69, 34.73 and 35.67 when tested on the TaqPath COVID-19 Combo kit and repeated negative when tested on SAMBA III. The remaining 143 concordant positive samples had Ct values when tested on the TaqPath COVID-19 Combo kit of 11.02 – 36.04.

Influenza A

In total, 39 Influenza A positive combined nose/throat samples collected in UTM from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, 28 were known to be H1N1, and 11 were known to be H3N2 based on testing at the reference laboratory from which these samples were acquired. When tested, all 39 were positive by SAMBA III. The Ct range for these samples, as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit, was 15.30 – 24.47.

Influenza B

In total, 13 Influenza B positive combined nose/throat samples collected in UTM from symptomatic individuals in Belgium were tested both by SAMBA III and TaqPath. Of these, all 13 were positive by SAMBA III. The Ct range for these samples, as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit, was 18.64 – 34.38.

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Respiratory Syncytial Virus (RSV)
In total, 57 RSV positive combined nose/throat samples collected in UTM from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, 28 were known to be RSV A, and 29 were known to be RSV B based on testing at the reference laboratory from which these samples were acquired. When tested, all 39 were positive by SAMBA III. The Ct range for these samples, as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit, was 11.61 – 36.06.

Negative Samples
In total, 214 negative combined nose/throat samples collected in UTM from asymptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 214 were negative for all targets by SAMBA III and TaqPath comparator kits.

Samples collected in SAMBA III Specimen Buffer

SARS-CoV-2
In total, 100 SARS-CoV-2 positive combined nose/throat samples collected in SAMBA III Specimen Buffer from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 100 were positive by SAMBA III. The Ct range for these samples, as determined by the TaqPath COVID-19 Combo kit, was 12.50 – 35.86.

Influenza A
In total, 29 Influenza A positive combined nose/throat samples collected in SAMBA III Specimen Buffer from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 29 were positive by SAMBA III. The Ct range for these samples as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit was 20.80 – 28.46.

Influenza B
In total, 8 Influenza B positive combined nose/throat samples collected in SAMBA III Specimen Buffer from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 8 were positive by SAMBA III. The Ct range for these samples as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit was 23.75 – 24.40.

Respiratory Syncytial Virus (RSV)
In total, 16 RSV positive combined nose/throat samples collected in SAMBA III Specimen Buffer from symptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 16 were positive by SAMBA III. The Ct range for these samples, as determined by the TaqPath COVID-19, FluA/B, RSV Combo kit, was 15.60 – 32.14.

Negative Samples
In total, 196 negative combined nose/throat samples collected in SAMBA III Specimen Buffer from asymptomatic individuals in the UK were tested both by SAMBA III and TaqPath. Of these, all 196 were negative for all targets by SAMBA III and TaqPath comparator kits.

Overall clinical performance
The overall sensitivity and specificity of SAMBA using TaqPath as a comparator test for each viral target is shown in Table 9 below.

In terms of clinical performance of the SAMBA III SCoV-2/Flu/RSV Test for SARS CoV-2 detection, the sensitivity and specificity of the assay in each of the individual collection buffer (SAMBA III Specimen Buffer and UTM) meet the CTDA requirements:

Collection buffer	(Sensitivity)	(Specificity)
SAMBA III Collection Buffer	100.00% (96.38-100.00)	100% (98.52-100.00)
UTM	97.95% (95% CI:94.11-99.57)	100.00% (95% CI: 98.86-100.00)

The overall invalid rate (IC failure) was 1.59% (13/818), and the overall technical failure rate (instrument mechanical or software failure) was 0.98% (8/818).

Table 9: Clinical performance of the SAMBA III SCoV-2/Flu/RSV Test compared to TaqPath COVID-19 test and TaqPath COVID-19, FluA/B, RSV Combo kit

Target	Sample buffer	No of specimens	+/+	+/-	-/-	-/+	PPA (95% CI)	NPA (95% CI)
SARS-CoV-2	UTM	469	143	0	323	3[1]	97.95% (94.11-100.00)	100.00% (98.86-100.00)
Flu A		469	39	0	430	0	100% (90.97-100.00)	100% (99.15-100.00)
Flu B		469	13	0	456	0	100% (75.29-100.00)	100% (99.19-100.00)
RSV		469	57	0	412	0	100% (93.73-100.00)	100% (99.11-100.00)
SARS-CoV-2	SAMBA Specimen Buffer	349	100	0	249	0	100.00% (96.38-100.00)	100% (98.53-100.00)
Flu A		349	29	0	320	0	100% (88.06-100.00)	100% (98.85-100.00)
Flu B		349	8	0	341	0	100% (63.06-100.00)	100% (98.92-100.00)
RSV		349	16	0	333	0	100% (79.41-100.00)	100% (98.90-100.00)
SARS-CoV-2	***All samples***	***818***	***243***	***0***	***572***	***3[1]***	***98.78% (96.48-100.00)***	***100.00% (99.36-100.00)***
Flu A		***818***	***68***	***0***	***750***	***0***	***100% (94.72-100.00)***	***100% (99.51-100.00)***
Flu B		***818***	***21***	***0***	***797***	***0***	***100% (83.89-100.00)***	***100% (99.54-100.00)***
RSV		***818***	***73***	***0***	***745***	***0***	***100% (95.07-100.00)***	***100% (99.51-100.00)***

+/+ concordant positive, +/- SAMBA positive/comparator negative, -/- concordant negative, -/+ SAMBA negative/comparator positive

[1]Ct of samples negative by SAMBA III were 34.69, 34.73 and 35.67 when tested on TaqPath COVID-19 Combo kit

Equivalency in performance between fresh and frozen specimens

The above clinical performance data demonstrate that the SAMBA III SCoV-2/Flu/RSV Test exhibits high sensitivity across all target organisms and extremely high specificity, regardless of the specimen type tested. Additionally, the UK study comparing Universal Transport Medium (UTM) with the SAMBA III Specimen Buffer confirmed that the assay performs equally well in both buffer types. These findings validate the utility and high performance of the SAMBA III SCoV-2/Flu/RSV Test in combined nose and throat swabs, as well as nasopharyngeal/oropharyngeal swab specimens, whether collected in UTM or

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the inactivating SAMBA III Specimen buffer. These features and its analytical specifications meet the requirements for the intended purpose of the device.

D. Samples collected in SAMBA III Specimen Buffer and tested with the SAMBA III SCoV-2/Flu/RSV Test

Of the 30 samples collected in the SAMBA III Specimen Buffer, all the samples were positive for SARS-CoV-2 by the SAMBA III SCoV-2/Flu/RSV Test when tested fresh and after being frozen for 24 hours. The samples were also tested using the TaqPath COVID-19, Flu A/B, RSV Combo kit and TaqPath COVID-19 Combo kit, and results were concordant with the SAMBA III SCoV-2/Flu/RSV Test. The TaqPath COVID-19 Combo kit averaged Ct values of the SARS-CoV-2 positive samples ranged from 27.76-33.61.

E. Samples collected in UTM and tested with the SAMBA III SCoV-2/Flu/RSV Test

Of the 30 samples collected in UTM, all the samples were positive for SARS-CoV-2 by the SAMBA III SCoV-2/Flu/RSV Test when tested fresh and after being frozen for 24 hours. The samples were also tested using the TaqPath COVID-19, Flu A/B, RSV Combo kit and TaqPath COVID-19 Combo kit, and the results were concordant with the SAMBA III SCoV-2/Flu/RSV Test. The TaqPath COVID-19 Combo kit averaged Ct values of the SARS-CoV-2 positive samples ranged from 11.02-20.49.

Shelf-life

Based on accelerated temperature study, the shelf life of the kit has been assigned at 6 months when stored at 2-30ºC. Its predecessor assay, SAMBA II SCoV-2/Flu/RSV Test has a shelf life of 12 months at 2-30°C.

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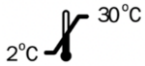
REVISION HISTORY

Version no.	Brief description of changes	Date approved
5.0	Updated intended use statement to be consistent with IVDR version. Updated Specimen collection and handling instruction to include details regarding storing and processing specimen samples. Updated Performance evaluation section for LoD to update reference to analytical sensitivity to LoD. Updated to cite references throughout IFU. Minor formatting changes throughout. Updates made for clarity and completeness, thus, risk is low.	03 June 2026
4.0	Updated UK rep address to First Floor, Park Central, 40-41 Park End Street Oxford OX1 1JD England, United Kingdom. Corrected code number for accessories listed on page 3 from C19-0130 to C19-0130-12 and C20-0009 to C20-0009-12 as materials are provided in a 12 pack to match the number of tests in a kit. Added UKCA mark to symbols description on page 44. Updates made for completeness, thus, risk is low.	20 August 2025
3.0	Added details on safety symbols on pages 3-5 and page 45, updated UTM claims on page 6 and 37; simplified Tablet result interpretation for RSV/FluB/FluA/SARS CoV-2 on pages 22-24; removed specimen storage claims at 2-8°C and 30°C on page 37, pending clinical sample stability study ; added UTM details on clinical SARS CoV-2 specimens tested on page 39; updated symbols on page 45 ; updated manufacturer address on page 46.	25 July 2025
2.0	Updated IFU throughout to correct minor types, grammatical errors and formatting issues. Updates made for clarity and completeness, thus risk is low.	18 November 2024
1.0	Initial release	07 November 2024

SYMBOLS	Description of Symbol
	Catalogue number
	Batch or Lot code
	**Store at 2-30ºC (Temperature limit)**
	***In vitro* diagnostic medical device**
	Use-by-date (YYYY-MM)
	Consult instructions for use
	Do not use if package is damaged
	Do not re-use
	Warning: (see Safety Data Sheet (SDS) for additional details)
	Danger: (see Safety Data Sheet (SDS) for additional details)
	Manufacturer
	UK Responsible Person who acts on behalf of the non-UK manufacturer in the UK.
	UKCA marking for United Kingdom (except Northern Ireland)
	Keep dry
	Fragile, handle with care
	This way up
	Contains sufficient for <*n*> tests

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