



Technical Validation for the Real World, SAMBA II SARS-CoV-2 Test (REF 8500-12)

Published: November 2020 (Version 2.0)

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Assay description and intended purpose

1. The SAMBA II SARS-CoV-2 Test is a nucleic-acid based amplification test for the qualitative detection of nucleic acid from the SARS-CoV-2 in human respiratory specimens (nose and throat swabs/ nasopharyngeal swabs/oropharyngeal swabs). Samples can be collected in virus transport medium or propriety Samba II inactivation buffer. Flocked, rayon or Dacron swabs with plastic shaft are suitable for collection. Swab specimens may be held at 2 to 30°C for up to 18 hours prior to testing. For refrigerated swab specimens, allow the sample to equilibrate to room temperature prior to testing. Freezing of samples should be avoided.
2. The assay is intended for use by professionals and trained operators on the SAMBA II instrument system in clinical point of care settings
3. The SAMBA II SARS-CoV-2 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. Don appropriate PPE according to your institution's health and safety requirements. Each propriety sample collection tube contains 2 ml of SAMBA Lysis Buffer which contains a detergent that inactivates the virus. A room temperature incubation period of 10 minutes is required

Type of sample to be used in validation

1. The validation was performed on dual collected nose and throat swabs or nasopharyngeal swabs collected in Samba II buffer and Viral transport medium. The samples were collected in clinical settings.
2. The Samba II is a fully automated method. Following addition of test cartridges and sample material to the Samba II SCoV Buffer (Code 8500E) onto the Samba II instrument, extraction amplification and detection of the Nucleic acid take place without further human intervention.

Equipment and reagents

All the equipment required is supplied by the manufacturer except for flocked, rayon or Dacron swabs with plastic shaft for sample collection with calibration/ service requirements and dates where applicable.

Performance characteristics

1. Analytical Sensitivity of SARS COV-2 targets

Lowest Limits of Detection (LLOD): Dilution series: Panel members for determination of the limit of detection (LOD) were prepared by making serial dilutions of SARS-CoV-2 RNA from strain 2019-nCoV/Italy-INMI1 from EVAg (code number: 008N-03894) in pooled negative combined nose and throat swab samples to target concentrations of 750, 500, 250, 200, 150 and 100 copies/ml. The initial LOD was determined by testing 6 levels at target concentrations of 750, 500, 250, 200, 150 and 100 copies/mL. Each panel member was tested in replicates of 3. The final LOD was confirmed by testing 250 copies/mL in replicates of 20, of which all were detected. Therefore, the claimed LOD of the SAMBA II SARS-CoV-2 Test is 250 cp/mL.

2. Linearity and efficiency: Not applicable (qualitative assay).

Precision and robustness

1. Intra-assay precision: N/A (qualitative assays).
2. Inter-assay precision: N/A qualitative assay.
3. Repeatability: 30 contrived positive specimens at approximately 1xLoD (n=3), 2x LoD (n=17), 3x LoD (n=5), 5x LoD (n=3) and 100x LoD (n=2) were tested. Samples were contrived by spiking known concentrations of SARS-CoV-2 RNA (2019-nCoV/Italy-INMI1 from EVAg) into individual negative patient specimens. The results are summarised in table 1.

Table 1: repeatability of controlled samples of known viral load

Concentration SARS-CoV-2 RNA (copies/mL)	Number tested	Number positive	% Positive
1-2x LoD	20	20	100 (n=20/20)
≥ 3x LoD	10	10	100 (n=10/10)
Negative	35	0	0 (n=0/35)

Analytical specificity (Interferences and cross-reactions)

1. A coronavirus RNA specificity panel containing 150 hCoV-NL63, hCoV-229E, hCoV151OC43 and MERS-CoV were sourced from the European Virus Archive, EVAg (code number 011N-03868). RNA samples from this panel were tested at >10,000 cp/test in the SAMBA II test in the presence or absence of SARS-CoV-2 RNA at 3x LOD (750 cp/ml) to determine specificity against other human coronaviruses. Other high-priority organisms indicated by the FDA EUA guidance document from manufacturers were sourced from Biodefense and Emerging Infections Research Resources Repository (BEI Resources) and American Type Culture Collection (ATCC) and tested in the presence and absence of SARS-CoV-2 RNA at 3x LOD (750 cp/ml) to evaluate the SAMBA II test for specificity and cross-reaction¹.
2. An in silico analysis for possible cross-reactions with related Coronaviruses was conducted by mapping primers in the SAMBA II SARS-CoV-2 Test individually to the sequences downloaded from the NCBI database. None of the SAMBA primers had >80% homology to the following related Coronaviruses: hCoV-NI63, hCoV-229E, hCoV-OC43, MERS-CoV and SARS-CoV). In addition, in silico analysis for possible cross-reactions with all the organisms in listed in the instructions for use was conducted by carrying out a BLASTn search for of each of the SAMBA primers and probes against NCBI databases and retrieving all sequences with homologies > 80%. Only one probe (N region) had greater than 80% homology (81%) to one of the high priority organisms (*Pneumocystis jirovecii* [PJP]). This marginal homology would not impact the performance of the test because the other primers and probes have no homology to *P. jirovecii*. This homology would not be able to compete with 100% homology of the SARS-CoV-2 amplicon to the capture probe.

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

1. Samples selected for the validation were appropriate to the assay. Low medium and high viral load samples were tested to avoid increasing or lowering diagnostic sensitivity and specificity.

Table 2: range of viral loads for validation samples

CT Range RNA Swab LAMP	CT <25	CT <33	Ct <45
Sample number (n)	89	47	28

2. Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) were compared. 164 positive samples were included to align with MHRA TPP (version 1.0). Clinical sensitivity (95% CI) and positive predictive value (PPV) was calculated in comparison with reference methods that had sensitivity and specificity and a limit of detection within the specifications of the MHRA TPP (Version 1.0). The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.
3. Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) were used. 261 negatives samples were included to align with MHRA TPP (Version 1.0). Clinical specificity (95% CI) and negative predictive value (PPV) was calculated in comparison with reference methods with sensitivity and specificity in line with the MHRA TPP (Version 1.0). The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.

Table 3: diagnostic sensitivity and specificity on Samba II

Report ID	CT <25	CT 25 to 30	Ct >30 to 35	Total positive	Total negative	Sensitivity	Specificity
Assennato et al ¹	48	22	6	76	26	98.7 (95% CI 92.89-99.97%),	100.0% (95% CI 87.23-100%),
Assennato et al ¹	6	6	3	15	55	100.0% (95% CI	100.0% (95% CI

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Report ID	CT <25	CT 25 to 30	Ct >30 to 35	Total positive	Total negative	Sensitivity	Specificity
						94.03-99.97%)	95.55-100%)
Collier et al ¹	12	14	7	33	116	97.0% (95% CI 84.2–99.9)	100% (95% CI 96.9–100)
FDA panel	23	5	12	40	64	100.0% 95% CI: 91.2% to 100.0%)	100.0% (95% CI: 94.4% to 100.0%),
Total				164	261	98.8%	100.0%

Additional Data

1. Local Verification reports

The Samba II assay has been locally verified in 6 NHS trust sites. Verification reports have been submitted to TVG for review

2. References

Performance evaluation of the SAMBA II SARS-CoV-2 Test for point-of-care detection of SARS-CoV-2, Sonny M Assennato, Allyson V Ritchie, Cesar Nadala, Neha Goel, Cuijuan Tie, Lourdes M Nadala, Hongyi Zhang, Rawlings Datir, Ravindra K Gupta, Martin D Curran, Helen H Lee *Journal of Clinical Microbiology* Oct 2020, JCM.01262-20; DOI: 10.1128/JCM.01262-20

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