ANALYTICAL METHOD VALIDATION

Capital Diagnostics Validation

report for

STANDARD™ Q COVID-19 Ag Test KIT

(SD BIOSENSOR)

Document number:
CD001/21 Version 1.0

ANALYTICAL METHOD VALIDATION and REPORT

STANDARD™ Q COVID-19 Ag Test **SD BIOSENSOR**

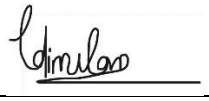
CAPITAL DIAGNOSTICS **DOCUMENT NO.: CD001/21 v1.0**

Contents

1 DOCUMENT APPROVAL

Section 1: Prepared by

The signature listed below, indicate the colleague responsible for the preparation of this document.

Company	Name	Designation	Signature	Date
Capital Diagnostics	Theolan Adimulam	Quality/Validation		10 Dec 2021

Section 2: Reviewed and Approved by

The signature listed below, indicate the colleague responsible for the review of this document.

Company	Name	Designation	Signature	Date
Capital Diagnostics	Veron Ramsuran	Senior Scientist		10 Dec 2021

2 INTRODUCTION

2.1 BACKGROUND

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and many countries including the United States. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported. As the COVID-19 epidemic rapidly spread across the world, South Africa was not spared. The country was forced to rapidly mobilise all resources in preparation for an upsurge of infections. These resources included those of patient care as well as diagnostic tests. In view of the COVID-19 epidemic, the seriousness of this virus and the urgency to perform laboratory testing, a team of scientists, diagnostic persons, pathologists and support structure was used to set up a high throughput COVID-19 Molecular laboratory as part of this COVID-19 rapid response. New variants have become to emerge. Recently, a new variant omicron has been detected within South Africa. It is unknown how existing COVID-19 testing kits perform against this new variant. For this reason SD BIOSENSOR approached CAPITAL DIAGNOSTICS to run a validation against samples with the omicron variant.

CAPITAL DIAGNOSTICS partners with other research labs such as Global Health Innovations (GHI), a subsidiary of Aurum Institute, in collaboration with the University of KwaZulu-Natal, engaged with personnel who had the experience to materialise and implement a highly professional and trusted service. This partnership allowed the recruitment of more omicron positive samples.

2.2 PURPOSE

The purpose of the method validation study is to demonstrate that the STANDARD™ Q COVID-19 Ag Test from SD BIOSENSOR is effective and suitable for its intended use as a COVID-19 antigen detection method for the detection of SARS-CoV-2 omicron variant.

2.3 SCOPE

The validation study will demonstrate:

- Method suitability and performance of STANDARD™ Q COVID-19 Ag Test from SD BIOSENSOR as a COVID-19 antigen detection method for use on SARS-CoV-2 omicron variant positive patients
- The validation will compare against gold standard PCR kit from ThermoFisher (TaqPath

COVID-19 RT-PCR Kit)

3 REFERENCE DOCUMENTS

- STANDARD™ Q COVID-19 Ag Test from SD BIOSENSOR. Cat. No. 09COV30D.

4 METHOD VALIDATION TEST PLAN

Validation testing compares the STANDARD™ Q COVID-19 Ag Test from SD BIOSENSOR with the TaqPath COVID-19 CE-IVD RT-PCR kit. A total of 30 omicron COVID-19 positive samples (as determined by the *TaqPath RT-PCR kit or NGS sequencing), and 10 COVID-19 negative samples was used to assess the variability in detection of SARS-CoV-2 and suitability of the antigen method for the detection of omicron positive patients. The assay was performed as per manufacturer's instructions and recommended run parameters. In order to confirm the omicron variant was detected by the TaqPath COVID-19 CE-IVD RT-PCR Kit, a total of three samples were Next Generation Sequenced and the online Basic Local Alignment Search Tool (BLAST) was used to validate the sequencers to omicron variants (see report below).

*S-gene advantage for potential early identification of the Omicron variant (B.1.1.529). This test uses a multi-target design that includes an S-gene target. If a sample with the 69-70del S-gene mutation is tested using the TaqPath COVID-19 CE-IVD RT-PCR Kit, it will result in an S-gene dropout. The European CDC and WHO have noted this pattern of detection may help with early identification of the Omicron variant (B.1.1.529).

5 VALIDATION ACCEPTANCE

Acceptance of the method validation procedure as being complete will be dependent on the results of the various tests meeting their respective acceptance criteria. Where the acceptance criteria have not been met then a justification for acceptance of the test will be made or an action plan will be provided to correct the deficiency.

6 TEST EQUIPMENT AND REFERENCE STANDARD VERIFICATION

Complete the test equipment/instrument and reference standard list. Verify that all listed equipment/instrument is qualified and within calibration and reference standards are within expiry date. See examples below.

Table 1: Equipment

Test Equipment	Instrument Reference #	Make	Status/ Verification	Verification expiry	Pass/Fail
QuantStudio 5 Real-time PCR (96 well block)	272520444	Thermofisher Scientific	Calibrated	June 2022	Pass
Vortex	85940	Scientific Industries Inc.	N/A	N/A	Pass
Thermal Cycler	805S9201150	Applied BioSystems	Calibrated	Sept 2022	Pass

Table 2: Reference Controls / Master Mix

Ref Std Name	Ref Std Batch/Lot no.#	Ref Std Expiry Date
TaqPath COVID-19 CE-IVD RT-PCR Kit	2226316	30 Nov 2022

Comments: All consumables used were nuclease-free and within expiry.

7 RESULTS

7.1 Method Validation data

7.1.1 Sample comparison of NGS and TaqPath COVID-19 CE-IVD RT-PCR Kit
Three reference samples were obtained from the University of KwaZulu-Natal Laboratory to perform a comparison verification on the presence of the SARS-CoV-2 omicron variant. Next generation sequencing using the Illumina miSeq was compared to the TaqPath COVID-19 CE-IVD RT-PCR Kit. All three samples had S gene dropouts from the Real-time PCR assay. The NCBI Blast results from the NGS sequencing are shown in Table 3 below.

Table 3: NCBI BLAST report for SARS-CoV-2 Omicron variants from S-gene dropout from TaqPath COVID-19 CE-IVD RT-PCR Kit

Sample ID	TaqPath COVID-19 CE-IVD RT-PCR Kit				**Sequencing result (blast site: https://www.ncbi.nlm.nih.gov/)**	**Pass/Fail**
	ORF1ab	N Gene	S Gene	IC	Top hit (% Percent Identity)	
CD015678	22.3	24.6	N/A	24.8	K032228/2021|EPI_ISL_6699754 |2021-11-19 (99.89%)	Pass
CD015699	18.9	19.2	N/A	22.9	K032241/2021|EPI_ISL_6699761 |2021-11-20 (99.91%)	Pass
CD015709	19.9	20.4	N/A	23.8	K032228/2021|EPI_ISL_6699754 |2021-11-19 (99.89%)	Pass

7.1.2 Comparison of STANDARD™ Q COVID-19 Ag Test from SD BIOSENSOR with the TaqPath COVID-19 CE-IVD RT-PCR kit
A total of 40 samples; 10 SARS-CoV-2 Negative (figure 1) and 30 SARS-CoV-2 omicron variant positive figure 2 and 3) were selected for comparison based on TaqPath COVID-19 CE-IVD RT-PCR kit results.

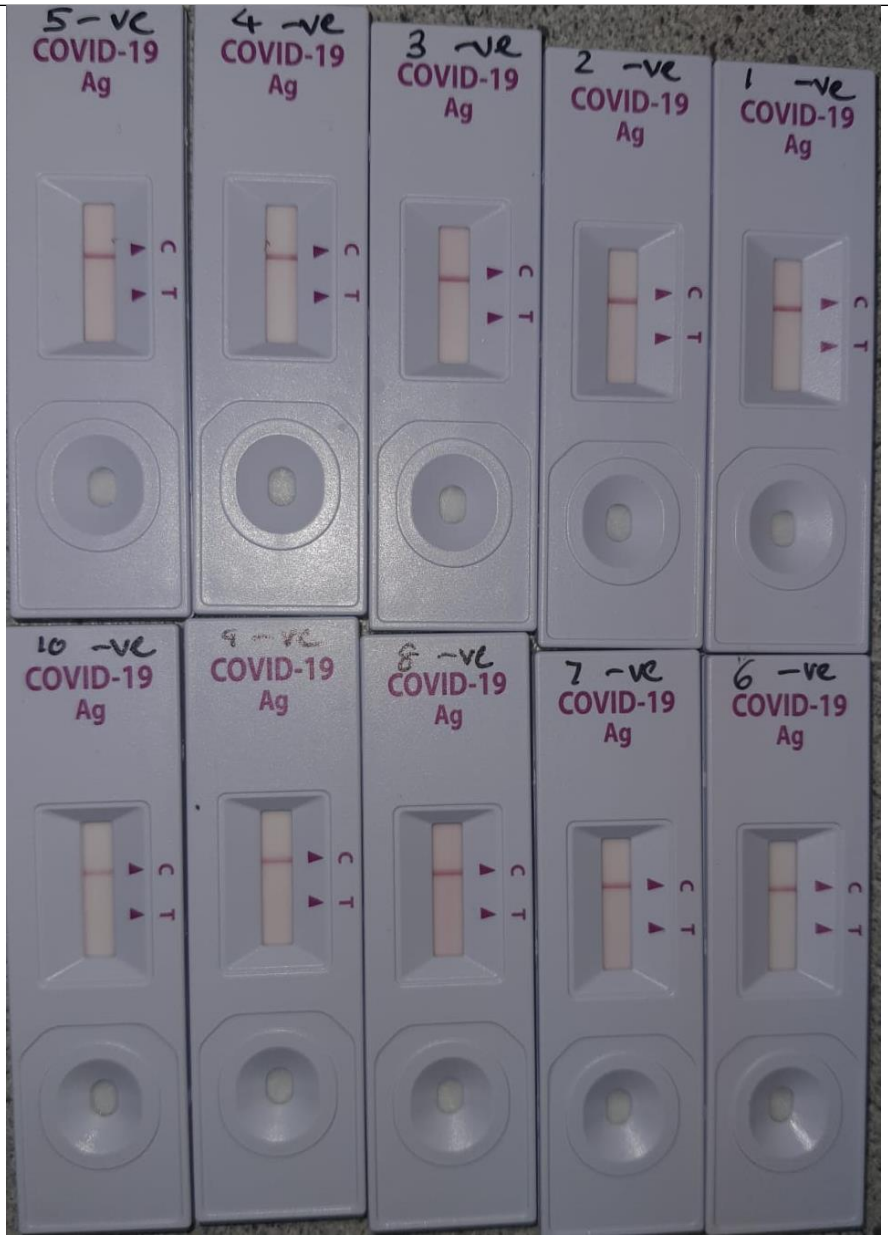


Figure 1. Ten SARS-CoV-2 Negative samples (as determined by TaqPath COVID-19 CE-IVD RT-PCR kit) run on the STANDARD™ Q COVID-19 Ag Test.

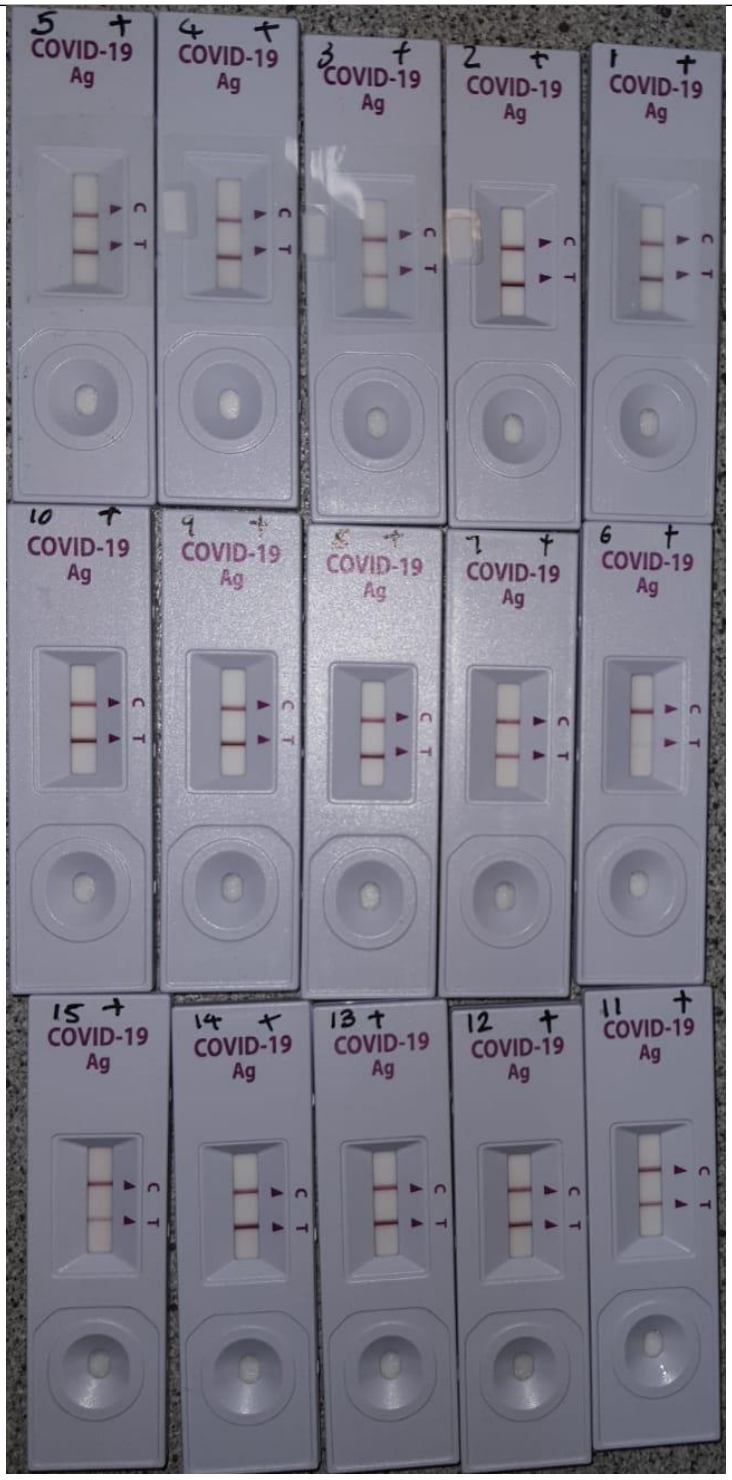


Figure 2. Fifteen SARS-CoV-2 Omicron Positive samples (as determined by TaqPath COVID-19 CE-IVD RT-PCR kit) run on the STANDARD™ Q COVID-19 Ag Test.

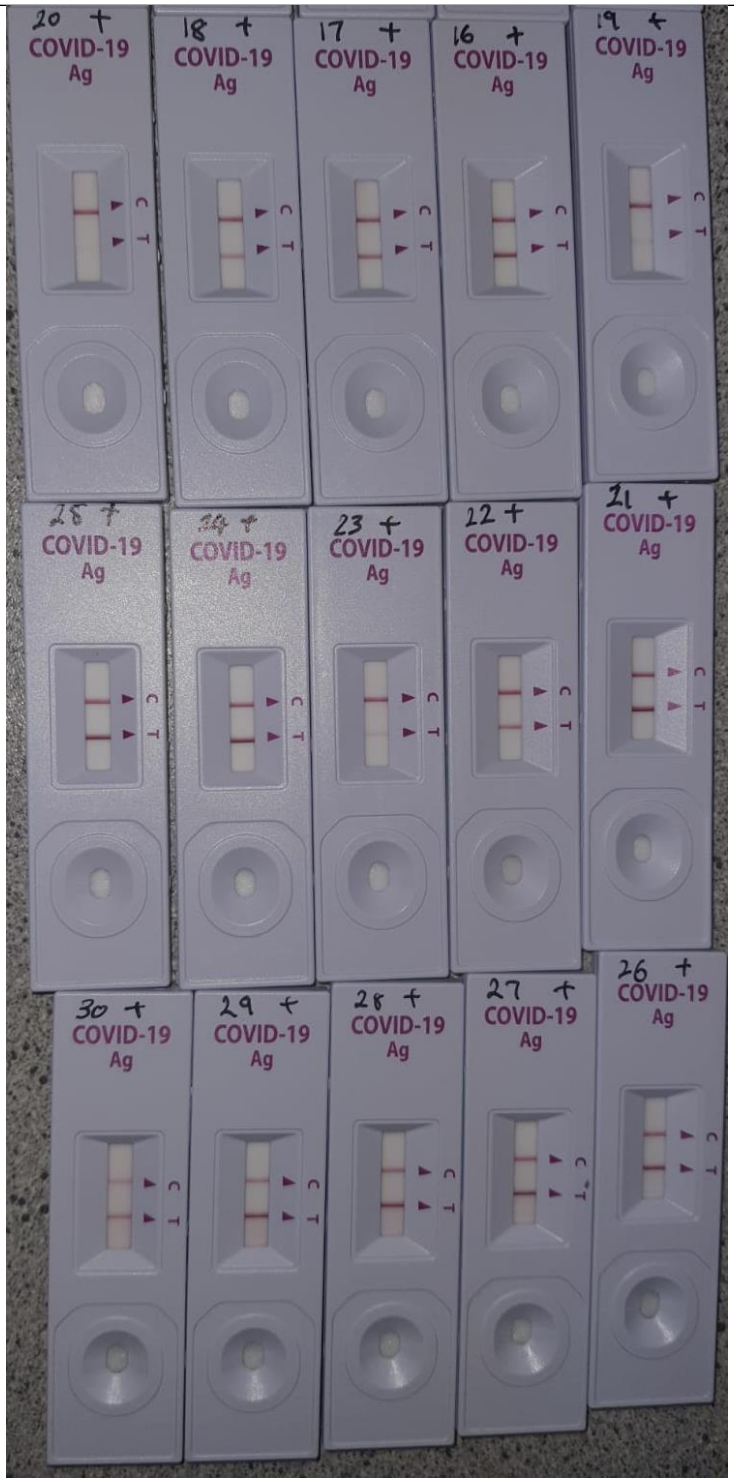


Figure 3. Fifteen SARS-CoV-2 Omicron Positive samples (as determined by TaqPath COVID-19 CE-IVD RT-PCR kit) run on the STANDARD™ Q COVID-19 Ag Test.

A head to head comparison of nasopharyngeal swabs from SARS-CoV-2 Omicron variant positive patients using the TaqPath COVID-19 CE-IVD RT-PCR Kit against the STANDARD™ Q COVID-19 Ag Test kits revealed a **96.7% sensitivity** (Table 4).

Table 4: Comparison of SARS-CoV-2 Omicron variant positive patients using TaqPath COVID-19 CE-IVD RT-PCR Kit and this STANDARD™ Q COVID-19 Ag Test on 40 samples

Sample ID	TaqPath COVID-19 CE-IVD RT-PCR Kit				STANDARD™ Q COVID-19 Ag Test	Result
	ORF1ab	N Gene	S Gene	IC	Positive/Negative	Pass/Fail
Neg 1	N/A	N/A	N/A	23.6	Negative	Pass
Neg 2	N/A	N/A	N/A	22.9	Negative	Pass
Neg 3	N/A	N/A	N/A	21.3	Negative	Pass
Neg 4	N/A	N/A	N/A	22.9	Negative	Pass
Neg 5	N/A	N/A	N/A	26.3	Negative	Pass
Neg 6	N/A	N/A	N/A	26.6	Negative	Pass
Neg 7	N/A	N/A	N/A	21.6	Negative	Pass
Neg 8	N/A	N/A	N/A	20.8	Negative	Pass
Neg 9	N/A	N/A	N/A	24.9	Negative	Pass
Neg 10	N/A	N/A	N/A	25.3	Negative	Pass
1	22.3	24.6	N/A	24.8	Positive	Pass
2	18.9	19.2	N/A	22.9	Positive	Pass
3	19.9	20.4	N/A	23.8	Positive	Pass
4	17.3	16.5	N/A	22.2	Positive	Pass
5	16.1	15.9	N/A	23.6	Positive	Pass
6	25.3	24.6	N/A	25.6	Positive	Pass
7	15.9	15.8	N/A	20.3	Positive	Pass
8	21.3	22.5	N/A	24.3	Positive	Pass
9	21.7	22.8	N/A	2	Positive	Pass

10	19.3	18.8	N/A	23.2	Positive	Pass
11	21.3	20.4	N/A	25.3	Positive	Pass
12	20.0	21.6	N/A	24.3	Positive	Pass
13	18.9	19.4	N/A	23.6	Positive	Pass
14	22.3	22.7	N/A	23.6	Positive	Pass
15	24.3	23.9	N/A	24.1	Positive	Pass
16	17.5	16.8	N/A	23.1	Positive	Pass
17	19.5	18.6	N/A	20.3	Positive	Pass
18	21.8	23.9	N/A	22.3	Positive	Pass
19	24.9	24.2	N/A	23.6	Positive	Pass
20	25.6	25.9	N/A	25.3	Negative	Fail
21	22.8	22.6	N/A	23.6	Positive	Pass
22	17.5	18.9	N/A	22.4	Positive	Pass
23	23.9	24.6	N/A	25.3	Positive	Pass
24	23.1	21.3	N/A	25.9	Positive	Pass
25	21.6	23.6	N/A	24.9	Positive	Pass
26	19.6	19.1	N/A	26.5	Positive	Pass
27	16.2	18.6	N/A	23.6	Positive	Pass
28	21.1	22.4	N/A	26.0	Positive	Pass
29	21.7	23.6	N/A	22.6	Positive	Pass
30	19.8	18.7	N/A	23.6	Positive	Pass

8 ANALYTICAL METHOD VALIDATION SUMMARY REPORT

Analytical Method validation Protocol Summary		
Ref. No.	**Test Data Sheet Title**	**Result (Pass/Fail)**
7.1	Analytical Method Validation Data	Pass
7.1.1	Method Suitability and Performance	Pass
7.1.2	Method Variability	Pass

Analytical Method Validation Report Summary	
Method Name	**Type**
STANDARD™ Q COVID-19 Ag Test	Antigen detection of SARS-CoV-2

Validation Parameter	Requirements	Results (Approved / Rejected)	Pass/ Fail
Method Suitability	STANDARD™ Q COVID-19 Ag Test is suitable for its intended purpose.	Approved	Pass
Method Performance	STANDARD™ Q COVID-19 Ag Test meets required performance criteria.	Approved	Pass
Method Variability	Limited / insignificant variability observed	Approved	Pass

Statement of Conformance:	
√	The validation for this test method have been successfully completed.
N/A	Notifications were raised due to discrepancies noted during execution. These have been resolved or have been noted for further action before closure of the validation report.

9 DISCUSSION AND CONCLUSION

All results were consistent with reference sample data, that is, limited/insignificant variability was observed with samples using the TaqPath COVID-19 CE-IVD RT-PCR Kit and STANDARD™ Q COVID-19 Ag Test. The STANDARD™ Q COVID-19 Ag Test meets the required specifications and is suitable for its intended use.

All data obtained within this validation study met required specification and no deviations or non-conformances were observed. The STANDARD™ Q COVID-19 Ag Test met all requirements and is suitable for use as detection of SARS-CoV-2 omicron variant positive patients. The method and instrument in place is efficient in terms of accuracy, precision, repeatability, specificity and sensitivity therefore meets user requirements based on manufacturer's guidelines as previously validated.

In order to ensure high quality of COVID-19 samples, it is recommended that for long storage, all reagent be stored at -20 °C to preserve integrity of the reagent and to avoid freeze thawing more than three times. Regular maintenance of the instrument is recommended, however based on usage, a planned preventative maintenance of the instrument may be arranged with the supplier. This is user specified. All reagents are stored at the relevant storage conditions based on manufacturers recommendations and used prior to expiration. All consumables used are sterile and nuclease free. Proper Personal protective equipment (PPE) is available on site and must be worn by all personnel on site at all times. Good laboratory practices are adhered to and maintained throughout all the facility.

----------End of Report----------

10 FINAL REPORT APPROVAL FORM

This is to certify that the undersigned have reviewed this executed protocol and have found that all applicable requirements and criteria have been met.

Section 1: Reviewed and Approved by

Company	Name	Designation	Signature	Date
Capital Diagnostics	Prof Veron Ramsuran	Senior Scientist		10 December 2021