QuikPath SARS-CoV-2 Diagnostic Kit

Instructions for Use (IFU)

For Research Use Only

25 Tests

Storage conditions:

RUO

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Disclaimer:

This assay has received Provisional Authorisation from the Health Sciences Authority in Singapore[2].This Test Kit may solely be used for research use or evaluation purpose and not for clinical purposes. This Test Kit may not be resold or used for any other purpose. Any intentional or unintentional use will not hold QuikPath Pte. Ltd. liable.

Table of Contents

1. INTENDED USE

QuikPath SARS-CoV-2 Diagnostic Test Kit is a single-use test kit intended for qualitative and visual detection of coronavirus SARS-CoV-2 viral RNA. This test uses nasopharyngeal and oropharyngeal swab specimens collected from patients suspected of COVID-19 by their healthcare provider.

Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab specimens during the acute phase of infection. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection 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.

Testing with the QuikPath SARS-CoV-2 Diagnostic Test Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

QuikPath SARS-CoV-2 Diagnostic Test Kit is intended for use in Singapore under the Health Sciences Authority (HSA) Provisional Authorization.

2. SUMMARY AND EXPLANATION

Coronavirus disease 2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. SARS-CoV-2 is a single-stranded, positive-sense RNA virus which is capable of person-to-person transmission and can cause mild to severe respiratory illness including death.

QuikPath SARS-CoV-2 Diagnostic Test Kit is a molecular in-vitro diagnostic test utilizing isothermal polymerase chain reaction (PCR) and colorimetric dye-based sensing for the qualitative and visual detection of the coronavirus SARS-CoV-2 viral RNA. In this document, we have provided pertinent and timely information about the QuikPath SARS-CoV-2 Diagnostic Test Kit.

3. PRINCIPLE OF THE TEST

QuikPath SARS-CoV-2 Diagnostic Test Kit is a Nucleic Acid Amplification Test (NAAT) for detection of SARS-CoV-2 viral RNA. It uses Reverse transcriptase Loop-mediated isothermal amplification in a sample-to-result cartridge format, wherein the presence of the virus results in a colour change of the reaction from pink to yellow after 40

minutes incubation. The test provides a qualitative positive or negative result at the end of the reaction based on the colorimetric readout which is detected by eye.

To perform the test, nasopharyngeal or oropharyngeal swab specimens are added to the buffer chamber in the QuikPath Test Cartridge to solubilize the sample. Both the pistons of the Test Cartridge are then pulled up to the maximum height and plunged back down to the bottom. This transfers an aliquot of the buffer into both the tubes of the Test Cartridge which will amplify any existing viral RNA. The Test Cartridge contains buffer, enzymes, and colorimetric reagents, necessary for 4 steps in the assay. These 4 steps are: Lysis of the virus, Reverse transcription of viral RNA to cDNA, Nucleic acid amplification, and Detection.

A heat block set at 65 °C is required to run the reaction for 40 minutes. After 40 minutes, the test results are interpreted by visualization of colour of the reaction in the two tubes of the Test Cartridge.

4. REAGENTS AND MATERIALS PROVIDED

Each Kit box contains:		
Item	**Description**	**Quantity**
Test Cartridges	Individually sealed in pouches	25**
Package Insert	Quick Instructions for use	1
IFU	Detailed Instructions for use	1
SDS	Safety Data Sheet	1
CoA	Certificate of Analysis	1

**Quantity of Test Cartridges in the Sample kits may vary as per the customer's order.

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

- Heat block maintained at 65°C for 40 minutes (Provided by QuikPath upon request)
- Gloves and PPE required to collect the sample
- Waste disposal
- Specimen Collection Swabs

Note: For optimal performance, use the Specimen Collection Swabs recommended by CDC[3]. Other swabs are not suitable for use with this test.

6. STORAGE AND HANDLING

- Test cartridges are shipped at 2- 4 °C and stored at -20°C based on manufacturer recommendations.
- Do not reuse the kit contents.
- Do not reuse nasopharyngeal or oropharyngeal Specimen Collection Swabs.
- Remove the Test Cartridge from storage and allow it to equilibrate to room temperature for 5 minutes before use.
- Do not use kit or reagents past the expiration date.
- Sample stability when using QuikPath SARS-CoV-2 Diagnostic Test Kit has been established for temperatures between 2- 4 °C for 24 hours.
- For best results, specimen collection swabs should be tested immediately after collection. If immediate testing is not possible, swabs can be stored in its original packaging at room temperature (15 °C to 30°C, 59°F to 86°F) for up to 2 hours prior to testing or refrigerated at 2 °C to 4 °C and tested within 24 hours from the time of collection.

7. PRECAUTIONS

- QuikPath SARS-CoV-2 Diagnostic Test Kit is For Research Use Only.
- QuikPath SARS-CoV-2 Diagnostic Test Kit has received Health Sciences Authority provisional authorization in Singapore.
- This Test kit has been authorized only for the testing of swabs for detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Follow universal precautions when handling patient samples. All patient samples should be treated as if potentially infectious. Follow standard BSL-2 guidelines when working with patient samples. Use appropriate personal protective equipment.
- Do not write on the Test Cartridge except in the indicated area on the cartridge label for recording patient ID and test date.
- Leave the Test Cartridges sealed in pouch until just before use.
- Do not remove the lid of the Test Cartridge until immediately before use. Once the lid is removed, add Specimen sample immediately (within 5 minutes) and start testing. Once a sample is added and the lid is closed, the test has started.
- Do not use any kits with visible damage.
- Do not use kit components after their expiration date.
- Sample collection & handling procedures require specific training & guidance.
- All kit components are single-use items. Do not use with multiple specimens.

- Dispose of kit contents and patient samples according to all local, state, and federal regulations.
- To help obtain accurate results, follow all the instructions, and regard all precautions in this Instructions for use (IFU).
- Inadequate or inappropriate sample collection, handling, processing, and/or storage can yield inaccurate results.
- Do not use visually bloody or overly viscous samples.
- Due to the high sensitivity of the QuikPath SARS-CoV-2 Diagnostic Test Kit, contamination of work area with previous samples may cause false positive results.
- Do not dismantle or try to open the Test Cartridge after the test is complete.
- Clean heat block & surrounding surfaces after use with 70% Ethanol or bleach
- Use the **Results Interpretation table** and the **Colour chart** in this IFU to interpret test results accurately.

8. SPECIMEN COLLECTION AND HANDLING

Each test should be completed with an oropharyngeal or nasopharyngeal Specimen Collection Swab using one Test Cartridge. Proper sample collection is an important step for an accurate test result. Use the standard protocol as directed by the local governing body for the specimen collection and handling.

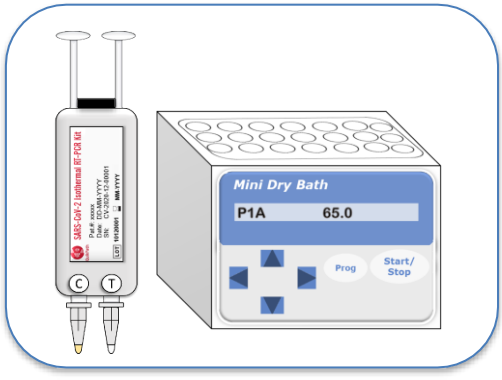
9. TEST PROCEDURE

- All clinical samples must be at room temperature before beginning the assay.
- Check the expiration date before using. Do not use test kit after expiry.

Note: The product images in this IFU are for illustration purposes only and may not be the exact representation of the product.

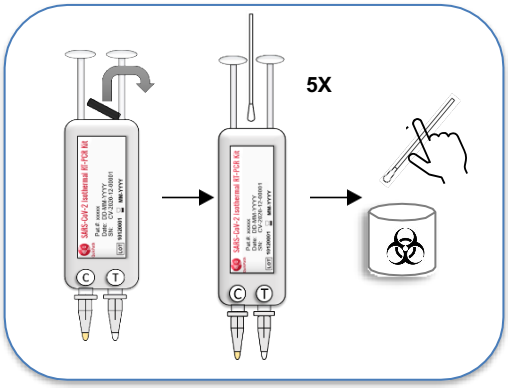
1. Initial Set- Up

- Remove Test Cartridge from the pouch.
- Let it equilibrate at room temperature for 5 minutes.
- Unpack the heat block, connect power cord to back of the unit, then plug it into a grounded outlet, turn the switch on.
- Press Start button. (**Note:** Display shows the default set programme P1A).
- Wait for the temperature to reach 65°C.
- Meanwhile, record the **Patient ID** and **Test date** on the Cartridge label.



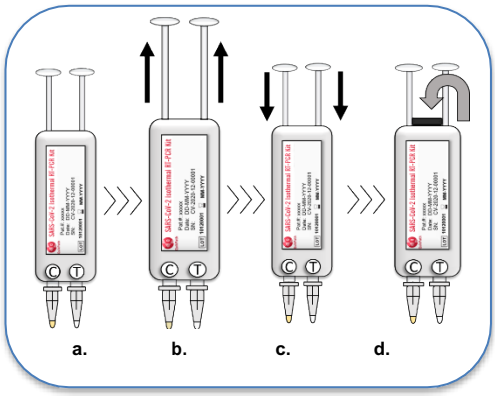
2. Swabbing and Cartridge Insertion

- Remove Test Cartridge lid & set aside. Insert the Specimen swab into the buffer chamber until it touches the bottom of the chamber.
- Rotate the swab **5x** in the chamber to dislodge the sample into the buffer.
- Remove Specimen swab from the buffer chamber and discard into biohazardous waste container.



3. Reaction activation

- From the initial position (Fig-a), pull both pistons upwards ~2cm (Fig-b), then immediately push down completely till the pistons do not move downwards any further (Fig-c). Volume of liquid in two tubes will increase if done correctly. Close the Cartridge lid, make sure it is secure (Fig-d).
- Record colour of liquid in two tubes (C & T) by comparing to **Colour Chart** below. This is called the **"Initial colour grade"**



- ➢ *If the colour grade is 3 to 5, proceed with the test.*
- ➢ *If colour grade is 1 or 2, test is invalid; re-start the test using a new cartridge.*

4. Run the Test

- Insert the Test Cartridge into the heat block for **40 minutes**, in the **red marked holes** only, as shown in Fig- 4(a).
- Push the Test Cartridge downwards to ensure tight fit (Fig-4(b)).
- Ensure both the tubes of the Cartridge are inserted into the heat block properly.

Note: Do not move the Test Cartridge and the heat block until the test is completed.

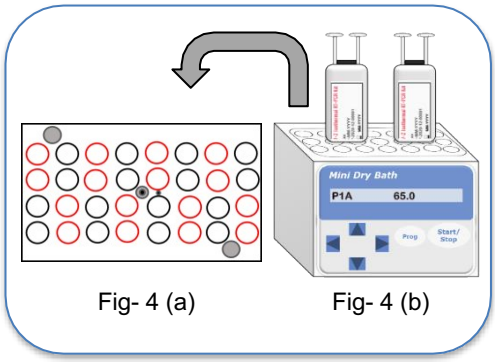
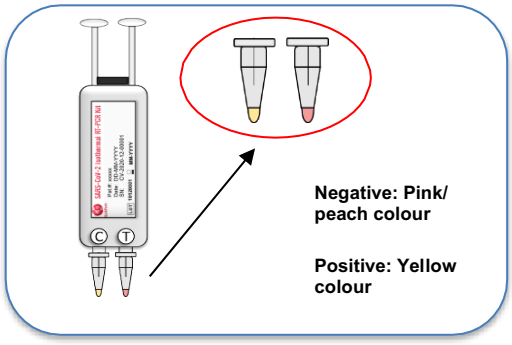


Fig- 4 (a) Fig- 4 (b)

5. Visualize the Result

- After 40 minutes, remove the Test Cartridge from the heat block.

- Let the Cartridge cool down for 5 minutes to room temperature.
- Observe colour of the reaction in the two tubes (C & T)
- Record the colour of liquid in two tubes (C & T) again by comparing to the **Colour Chart** below. This is called the **"Final colour grade".**
- Please refer to the tubes images in the **Results Interpretation table** below.



10. INTERPRETATION OF RESULT

Note: Look closely at the colour change in both tubes (C and T) to interpret the test results. Visualize the tubes in natural lighting. Individuals with colour-impaired vision may not be able to adequately interpret test results.

1) Refer to the **Colour Chart** below for steps to determine Positive and Negative Results. Also refer to the images in the **Results Interpretation table** below for test results validity interpretation.

Colour Chart

GRADE 1	GRADE 2	GRADE 3	GRADE 4	GRADE 5
				

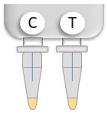
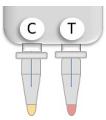
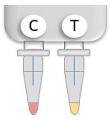
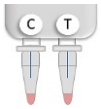
2) In the **Colour Chart**, find the difference between Initial colour grade (Test procedure- step 3) and final colour grade (Test procedure- step 5).

3) If, **(Final colour grade — Initial colour grade) >= 2: Test result is Positive**

If, **(Final colour grade — Initial colour grade) < 2: Test result is Negative**

Results Interpretation table

Note: In the below table, “+” symbol denotes positive, “-” symbol denotes negative.

Tubes Colour after Reaction	Internal Control Tube (C)	Test Sample Tube (T)	Interpretation
	+	+	Test result is valid. SARS-CoV-2 is detected
	+	-	Test result is valid. SARS-CoV-2 is not detected
	-	+	Test result is invalid. Sample should be re-tested
	-	-	Test result is invalid. Sample should be re-tested

Note: If an invalid result is obtained, the sample may be rerun. A new sample should be collected and run with a new Test Cartridge.

11. QUALITY CONTROL

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user’s laboratory’s standard quality control procedures. Quality control procedures are intended to monitor reagent and assay performance.

INTERNAL CONTROL: The Test Cartridge has an integrated Internal Control (labelled "C" on the cartridge) to validate the test results.

EXTERNAL POSITIVE AND NEGATIVE CONTROLS: External controls may be purchased separately. Please contact QuikPath for the technical information.

12. SAMPLE STORAGE AND SAMPLE EXTRACTION

- For best results, Specimen Swabs should be tested immediately after collection.
- If immediate testing is not possible, a swab can be stored in its original packaging at room temperature (15°C to 30°C, 59°F to 86°F) for up to 2 hours prior to testing.
- If a swab cannot be tested within 2 hours, it can be refrigerated at 2°C - 8°C and tested within 24 hours from the time of collection.

- Do not freeze the prepared sample prior to testing.
- Patient Swabs previously stored in viral transport media are not recommended and will invalidate the test.

13. LIMITATIONS

- The performance of the QuikPath SARS-CoV-2 Diagnostic Test Kit was determined using the procedures provided in this IFU. Failure to follow these procedures may alter test performance.
- The QuikPath SARS-CoV-2 Diagnostic Test Kit is for use with oropharyngeal or nasopharyngeal swab specimens. Improper collection, storage or transport of specimens may lead to false negative or invalid results.
- Test results should be interpreted in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests performed.
- As with other tests, negative results do not rule out SARS-CoV-2 infections and should not be used as the sole basis for patient management decisions.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- False negative or invalid results may occur due to interference or the presence of inhibitors.
- False negative results may occur if viruses are present at levels below the test's limit of detection.
- False negative results may occur if mutations are present in the regions targeted by the test.
- Cross-reactivity with respiratory tract organisms other than those listed in the Analytical Specificity Study may lead to erroneous results.
- This test cannot rule out diseases caused by other viral or bacterial agents.
- Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability. Detection of analyte targets does not imply that the corresponding viruses are infectious or are the causative agents for clinical symptoms.
- Laboratories are required to report all positive results to the appropriate public health authorities.

14. PERFORMANCE CHARACTERISTICS

a. Limit of Detection (LoD)- Analytical Sensitivity

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which ≥ 95% of all (true positive) replicates test positive. Limit of Detection (LoD) testing was performed with

NATtrolTM SARS-CoV-2 Stock (Isolate: USA- WA1/2020) purchased from Zeptometrix corporation. For each replicate, a contrived sample was prepared by pipetting 1 µL of the viral dilution into 99 µL of two diluents: 1) Buffer and 2) Pooled negative oropharyngeal and nasopharyngeal swab human clinical matrix. Three replicate samples were prepared for each dilution. The minimum dilution that produced all 3 positives was then subsequently run 20 times to establish the LoD. The dilution that reproducibly produced at least 19/20 (95%) positive results was confirmed as the LoD. The LoD was determined to be 10 copies/µL when tested in the human clinical matrix. This LoD was used when preparing samples for other performance testing. In the buffer, the LoD was determined to be 5 copies/µL. The data is summarized in the table below:

Table 1: LOD in contrived clinical sample

Copies/ µL	Replicate 1	Replicate 2	Replicate 3	Sensitivity %
10,000	Positive	Positive	Positive	100
1000	Positive	Positive	Positive	100
100	Positive	Positive	Positive	100
10	Positive	Positive	Positive	100
1	Negative	Negative	Positive	33

Table 2: Confirmation of LoD

RNA Diluted in pooled oropharyngeal & nasopharyngeal Swab Human Clinical Matrix	
Final Concentration	**Test Result**
10 copies per µL	19/20

b. Analytical Reactivity/ Inclusivity

Due to the limited availability of SARS-CoV-2 isolates for inclusivity testing, *in silico* analysis was employed to evaluate the extent of homology between QuikPath SAR-CoV-2 primers and all sequenced SARS-CoV-2 isolates available in the public databases (NCBI and GISAID). Table 3. below summarizes the homology between SARS-CoV-2 sequences and the QuikPath SARS-CoV-2 Test primers.

Table 3: Summary of Homology

Oligonucleotide	Homology
ORF1ab forward primer	99.9%
ORF1ab reverse primer	100%
N gene forward primer	99.9%
N gene reverse primer	100%

c. Analytical Specificity –Exclusivity (Cross Reactivity)

Table 4. below summarises the findings of *in silico* exclusivity analysis examining the homology between the indicated organisms and the QuikPath SARS-CoV-2 Test primers. Potential interactions are noted where primer homology exceeds 75%.

Table 4: Summary of in silico Exclusivity Analysis

List of organisms Analysed in-silico			
Other high priority pathogens from the same genetic family	**Result**	**High priority organisms likely in the circulating area**	**Result**
Human coronavirus 229E	no significant similarity found	Adenovirus (e.g., C1 Ad. 71)	no significant similarity found
Human coronavirus OC43	no significant similarity found	Human Metapneumovirus (hMPV)	no significant similarity found
Human coronavirus HKU1	no significant similarity found	Parainfluenza virus 1-4	no significant similarity found
Human coronavirus NL63	no significant similarity found	Influenza A & B	no significant similarity found
SARS-coronavirus	no significant similarity found	Enterovirus (e.g. EV68)	no significant similarity found
MERS-coronavirus	no significant similarity found	Respiratory syncytial virus	no significant similarity found
		Rhinovirus	no significant similarity found
		Chlamydia pneumoniae	no significant similarity found
		Haemophilus influenzae	no significant similarity found
		Legionella pneumophila	no significant similarity found
		Mycobacterium tuberculosis	no significant similarity found
		Streptococcus pneumoniae	no significant similarity found
		Streptococcus pyogenes	no significant similarity found
		Bordetella pertussis	no significant similarity found
		Mycoplasma pneumoniae	no significant similarity found
		Pneumocystis jirovecii	no significant

		(PJP)	similarity found
		Candida albicans	no significant similarity found
		Pseudomonas aeruginosa	no significant similarity found
		Staphylococcus epidermis	no significant similarity found
		Staphylococcus salivarius	no significant similarity found

In addition, a wet lab exclusivity study was performed by testing 12 potentially cross-reacting organisms with the QuikPath SARS-CoV-2 Test. Inactivated test cultures of all organisms were obtained from Zeptometrix corporation, USA. Each organism was diluted in a pooled negative human oropharyngeal swab and nasopharyngeal swab matrix and tested in triplicate. The organisms and results are shown in Table 5. None of the 12 organisms cross-reacted in the QuikPath SARS-CoV-2 Test at the concentrations tested.

Table 5: Summary of Wet Lab Exclusivity study

Organism	Result
SARS CoV	Negative
MERS CoV	Negative
Coronavirus OC 43	Negative
Coronavirus NL63	Negative
Coronavirus 229E	Negative
Coronavirus HKU1	Negative
Influenza A virus H1N1-2009	Negative
Influenza A virus H1N1	Negative
Influenza A virus H3N2	Negative
Influenza B virus	Negative
Respiratory Syncytial Virus (RSV)	Negative
Adenovirus type 1	Negative
Adenovirus type 3	Negative
Adenovirus type 31	Negative
C. pneumoniae	Negative
Human Metapneumovirus Type 8	Negative
M. pneumoniae	Negative
Parainfluenza Type 1	Negative
Parainfluenza Type 4	Negative
Rhinovirus Type 1A	Negative
B. parapertussis	Negative

B. pertussis	Negative
Parainfluenza Type 2	Negative
Parainfluenza Type 3	Negative

d. Analytical Specificity - Interfering Substances

To assess substances with the potential to interfere with the performance of the QuikPath SARS-CoV-2 Diagnostic Test Kit, contrived samples with SARS- CoV-2 RNA were tested in replicates of three (3) with each interfering substance at the “worst case” concentration, and negative samples without RNA were tested once with each interfering substance at the “worst case” concentration. The SARS-CoV-2 RNA was tested at 3X the LoD confirmed in the Limit of Detection Study described above. For each positive sample, RNA was diluted into a pooled negative nasopharyngeal and throat mix swab matrix to achieve a 3X LoD concentration.

The SARS-CoV-2 RNA was tested with an interferent concentration representing the highest concentration likely to be found in a respiratory or throat sample. Potentially interfering substances that were sourced in their solid phase were re-suspended and diluted to a concentration deemed to be likely worst case. Liquid phase potential interferents were not diluted before testing. Additionally, the SARS-CoV-2 RNA was tested without the interfering substance as a control. Potential interferents and their concentrations, samples tested, and test results are summarized in Table 6. No interference was observed with any of the substances tested.

Table 6: Summary of Interfering substances evaluation

Potential Interferent	Active Ingredient	Concentration Tested	Target	% Agreement with Expected Results
Blood	Blood	5%	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100
Mucin	Mucin bovine submaxillary gland, type I-S	50 mg/ml	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100
Nasal spray	Triamcinolone acetonide, Oxymetazoline HCl	10%	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100
Antibiotic	Amoxicillin	100 mg/ml	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100

			Negative	(1/1) 100
Mouthwash	Eucalyptol, Menthol,Methyl Salicylate, Thymol	10%	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100
Althos lozenges	Menthol, Eucalyptus, Thymol	1lozenge/ 5ml	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100
Alcohol	Ethanol	10%	Positive SARS-COV-2(3X LoD)	(3/3) 100
			Internal Control	(1/1) 100
			Negative	(1/1) 100

e. Clinical Evaluation (Contrived specimens)

Thirty (30) negative samples and 30 positive contrived samples were tested with the QuikPath SARS-CoV-2 Test. Negative samples were collected from consented healthy volunteers. Oropharyngeal swabs and nasopharyngeal swabs were collected from donors and eluted together in the buffer. Positive samples were prepared from these negative samples. Positive samples were spiked with SARS-CoV-2 inactivated virus (NATtrolTM SARS-CoV-2 Stock (Isolate: USA- WA1/2020)) at the concentrations shown in Table 7.

Table 7: Summary of Clinical Evaluation

LoD	N	Percent (%) Agreement with Expected Results (Observed/Expected)
2x	20	100 (20/20)
5x	7	100 (7/7)
10x	2	100 (2/2)
50x	1	100 (1/1)
Negative	30	100 (30/30)
Internal Control	30	100 (30/30)

15. ASSISTANCE AND CONTACT INFORMATION

For technical questions or assistance, or if the QuikPath SARS-CoV-2 Diagnostic Test Kit is not performing as expected, please contact QuikPath Pte. Ltd. at info@quikpath.sg or (+65) 93729144.

16. REFERENCES

1. (2020) Naming the coronavirus disease (COVID-19) and the virus that causes it [Online] WHO.
2. COVID-19 Diagnostic Tests that received HSA Approval in Singapore via Provisional Authorisation: https://www.hsa.gov.sg/announcements/regulatory-updates/hsa-expedites-approval-of-covid-19-diagnostic-tests-in-singapore-via-provisional-authorisation
3. CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html#specimen
4. World Health Organization Interim guidance on laboratory biosafety: www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratoryguidance from 13 May 2020

17. TABLE OF SYMBOLS

Symbol	Description
	Product is for single use only. It is not to be re-used
	Manufacturer information
	Refer the Instructions For Use (IFU)
	Product has a temperature limitation
	Use-by date
	Product batch code
	Contains Maximum number of Tests
	For Research Use Only
	Keep away from sunlight
	Do not use if package is damaged
	Keep away from rain