PHASE SCIENTIFIC
相達生物科技

INDICAID COVID-19 Rapid Antigen Test

TECHNICAL FILE

IDENTIFIER: TF 002

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2 General

2.1 Manufacturer and Authorized Representative

Legal Manufacturer	PHASE Scientific International 32 & 33/F, Gravity, 29 Hing Yip Street Kwun Tong, Kowloon, Hong Kong Phone:
Manufacturing Site(s):	PHASE Scientific International 32 & 33/F, Gravity, 29 Hing Yip Street Kwun Tong, Kowloon, Hong Kong Phone:
Authorized Representative	LOTUS NL B.V. Koningin Julianaplein 10 1e Verd, 2595AA The Hague The Netherlands

2.2 Design Facilities

Phase Scientific International

2.3 Manufacture Facilities

Facilities	Activities performed
PHASE Scientific International 32 & 33/F, Gravity, 29 Hing Yip Street Kwun Tong, Kowloon, Hong Kong Phone:	Finished Kit Assembly; Distributor; Product support, Sales and Marketing
PHASE Diagnostics 10527 Garden Grove Blvd. Garden Grove, CA 92843, USA Phone: 1 (657) 233-5880	US Distributor

2.4 Licenses and Registrations

Self-Declared CE Mark

2.5 Relevant Regulations

The firm's products are manufactured and distributed under the following regulations/standards:

US FDA GMP 21 CFR 820
98/79/EC In Vitro Diagnostic Medical Devices Directive
ISO 13485: 2016 (no certification)

2.6 Product Classification

Other IVD – other than Self-Test

2.7 Product Identification and Variants

Product name	Product Code
INDICAID COVID-19 Rapid Antigen Test – 25 test kit	2110200
INDICAID COVID-19 Rapid Antigen Test – 5 test kit	2120200
INDICAID COVID-19 Rapid Antigen Test – 1 test kit	2120100

2.8 Quality System Manual

The work instructions, standard operating procedures and other records associate with the design, production, release, and distribution of the devices is defined in the firm's Quality System Manual which is provided in Appendix A.

2.9 Conformity Assessment

In Vitro Diagnostics Directive 98/79/EC: IVD Professional: Annex III (section 6 excluded)

2.10 List of Applicable Standards and Essential Requirements

A list of the standards applicable standards to the design, production, and distribution of the firm's products has been identified and is maintained. This list of standards is provided in Appendix B.

The applicable sections of the essential requirements of the Directive(s) have been identified and are documented in the Essential Requirements Checklist. Rationale is provided for any requirement(s) that are deemed not applicable. This document is provided in Appendix C.

2.11 EC Declaration of Conformity

Refer to Appendix D for the EC Declarations of Conformity for the products listed in section 2.7.

3 Product Information

3.1 General Description

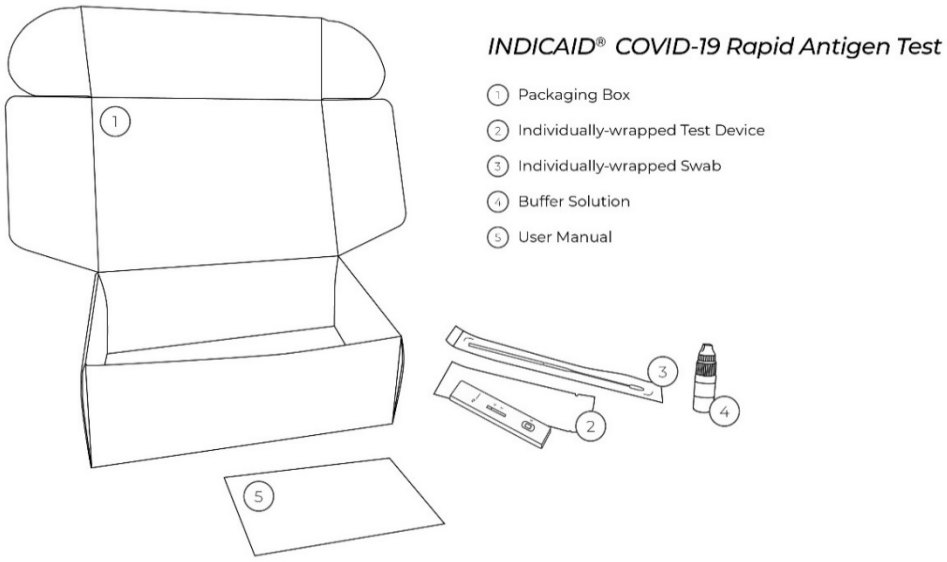
The INDICAID® COVID-19 Rapid Antigen Test is a lateral flow immunoassay intended for the qualitative detection of antigens specific to SARS-CoV-2 in direct nasal and nasopharyngeal swab samples from individuals who are suspected of COVID-19 by their healthcare provider. The INDICAID® COVID-19 Rapid Antigen Test is for use by medical professionals or trained operators.

3.2 Testing Principal Description and Illustration

The INDICAID® COVID-19 Rapid Antigen Test is an immunochromatographic membrane assay that uses highly sensitive antibodies to detect SARS-CoV-2 antigen from nasal and nasopharyngeal swab samples. SARS-CoV-2 specific antibodies and a control antibody are immobilized onto a nitrocellulose membrane support as two distinct lines. The test line (T) region contains monoclonal anti-SARS-CoV-2 antibodies, and the control line (C) region contains the control antibody. Monoclonal anti-SARS-CoV-2 antibodies conjugated with red colloidal gold particles are used to detect the SARS-CoV-2 antigen. During the test, the patient sample swab is placed in a Buffer Solution Vial. That Buffer Solution is then applied to the sample well of the test device. If SARS-CoV-2 antigen is present, it will bind to the antibody-gold conjugate forming an immunocomplex. The immunocomplex will then travel across the strip via capillary action towards the test line. The immunocomplex will then bind to the SARS-CoV-2 antibodies at the test line (T), forming a visible red line to indicate detection of antigen. If SARS-CoV-2 antigens are not present, no color will appear at the test line (T). Test results are interpreted at 20 minutes after application of the Buffer Solution to the Test Device. Results should not be read after 25 minutes. The control line is used for procedural control and should appear red regardless of the test result to ensure the test is performing properly.

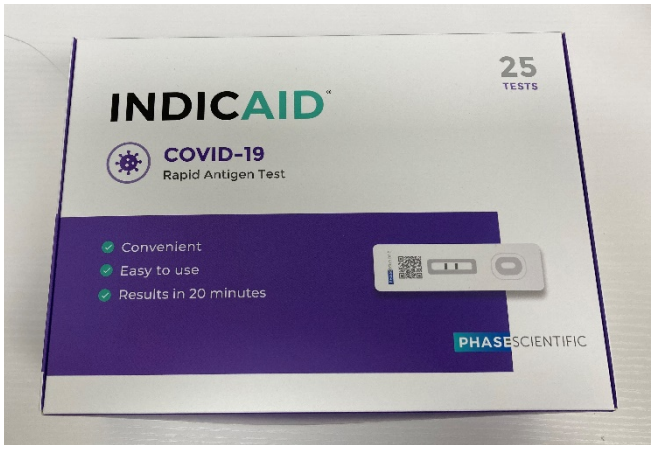
3.3 Test Format and Configuration

INDICAID COVID-19 Rapid Antigen Test 25 tests kit

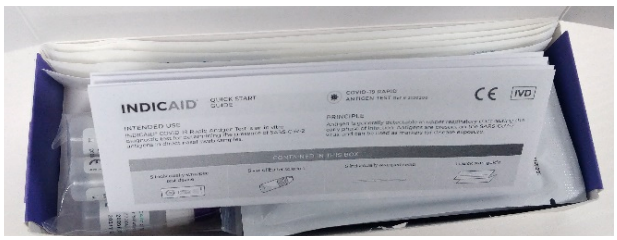


INDICAID® COVID-19 Rapid Antigen Test (25 reactions):

Component	Qty/kit
Individually-wrapped Test Device	25
Individually-wrapped Swab	25
Buffer Solution Bag	1
Buffer Solution	25
Instruction For Use	1



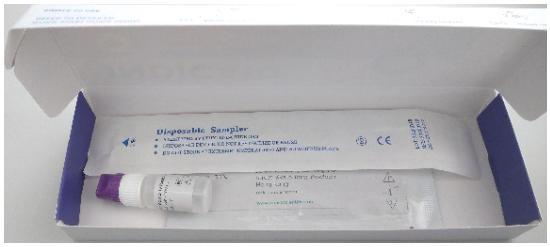
INDICAID COVID-19 Rapid Antigen Test – 5 test kit

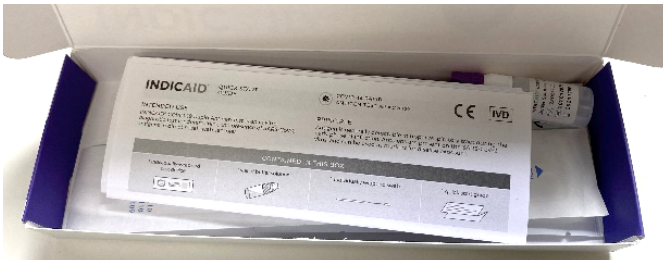


INDICAID® COVID-19 Rapid Antigen Test (5 reactions):

Component	Qty/kit
Individually-wrapped Test Device	5
Individually-wrapped Swab	5
Buffer Solution Bag	1
Buffer Solution	5
Product Insert	1

INDICAID COVID-19 Rapid Antigen Test – 1 test kit





INDICAID® COVID-19 Rapid Antigen Test (1 reaction):

Component	Qty/kit
Individually-wrapped Test Device	1
Individually-wrapped Swab	1
Buffer Solution	1
Product Insert	1

Refer to Appendix J for Device Master Record.

3.4 Drawing and Diagrams

<please insert ED drawing for cassettes, buffer vials, swab, and sample bag>

3.5 Labeling

Refer to Appendix F for product labels

- F1 - INDICAID COVID-19 Rapid Antigen Test Box – 25 test kit

Items	Dimension (L x W x H) (mm)
Box	165 x 225 x 80

- F2 - INDICAID COVID-19 Rapid Antigen Test Label – 25 test kit
- F3 - INDICAID COVID-19 Rapid Antigen Test Box (Printed Box) – 5 test kit

Items	Dimension (L x W x H) (mm)
Box	65 x 205 x 35

- F4 - INDICAID COVID-19 Rapid Antigen Test Box (Printed Box) – 1 test kit

Items	Dimension (L x W x H) (mm)
Box	65 x 205 x 25

- F5 - INDICAID COVID-19 Rapid Antigen Test Device Label (Printed Pouch)
- F6 - INDICAID COVID-19 Rapid Antigen Test Buffer Solution label

3.6 Instruction for Use

Refer to Appendix F7, F8 and G for User Manual (Instruction for Use) and Quick Start Guide

- F7 - INDICAID COVID-19 Rapid Antigen Test Quick Start Guide for 5 test kit
- F8 - INDICAID COVID-19 Rapid Antigen Test Quick Start Guide for 1 test kit
- G1 - INDICAID COVID-19 Rapid Antigen Test User Manual for 25 test kit
- G2 - INDICAID COVID-19 Rapid Antigen Test User Manual for 5 test kit
- G3 - INDICAID COVID-19 Rapid Antigen Test User Manual for 1 test kit

3.7 Risk Analysis

Refer to Appendix H for FMEA

Refer to Appendix I for Risk Management Report

3.8 Performance Analysis and Evaluation

Limit of Detection (LoD)

The purpose of this study is to establish the analytical sensitivity of the INDICAID® COVID-19 Rapid Antigen Test. This study determines the limit of detection (LoD) by using respiratory specimens collected from clinical sample matrix (mid-turbinate nasal swab samples) spiked with gamma-irradiated SARS-CoV-2 whole inactivated virus.

A 10-fold dilution series with three replicates per concentration were tested. Then, the results were be further refined with a follow-up study of two-fold dilutions between the last dilution to give three positive results and the first dilution to give at least one negative result. The lowest dilution with 100% positivity rate will then be tested with 20 replicates to confirm the final LoD concentration. The final LoD is defined as the lowest concentration at which 19/20 replicates are positive. One lot of test kits were used for the LoD range finding and confirmation studies. Three lots of test kits were used for the refining study.

For the initial LoD range finding study, 10-fold serial dilutions of gamma-irradiated SARS-CoV-2 in pooled human nasal matrix were prepared with the highest test concentration of 2.8 $x10^5$ $TCID_{50}$/mL (1.4 x 10^4 $TCID_{50}$/swab). From this dilution series, the lowest concentration to produce 3 out of 3 positive results on the INDICAID® COVID-19 Rapid Antigen Test was 2.8 $x10^3$ $TCID_{50}$/mL (1.4 x 10^2 $TCID_{50}$/swab). This tentative LoD was further refined using 2-fold serial dilutions between 2.8 $x10^3$ $TCID_{50}$/mL (1.4 x 10^2 $TCID_{50}$/swab) and 1.75 $x10^2$ $TCID_{50}$/mL (8.75 $TCID_{50}$/swab). From this 2-fold dilution series, a concentration of 2.8 $x10^3$ $TCID_{50}$/mL (1.4 x 10^2 $TCID_{50}$/swab) continued to be the lowest concentration that produced 3 out of 3 positive results. This concentration was confirmed to be the final LoD as 20 out of 20 replicates produced a positive result with test samples containing 1.4 x 10^2 $TCID_{50}$/swab.

For detailed testing procedures and raw data, please refer to Appendix K1 and M1.

Hook Effect

High-dose Hook Effect study was performed on the INDICAID® COVID-19 Rapid Antigen Test to determine the concentration at which false negatives are produced when very high level of target is present in a tested sample. Inactivated SARS-CoV-2 virus up to a concentration of 2.8 x 10^5 $TCID_{50}$/mL was used to spiked into real clinical matrix from SRS-CoV-2 negative donors.

3 operators will perform the study with five concentrations, 2.8 x 10^5, 2.8 x 10^4, 2.8 x 10^3, 2.8 x 10^2, and 2.8 x 10^1, and negative control sample. Each level was tested in triplicate.

All results from 3 operators are aligned where negative results were obtained at negative, 2.8 x 10^1, and 2.8 x 10^2. Positive results were obtained at 2.8 x 10^3, 2.8 x 10^4, 2.8 x 10^5. The results indicate that no hook effect was observed at 2.8 x 10^5 $TCID_{50}$/mL.

For detailed testing procedures and raw data, please refer to Appendix K1 and M1.

Specimen Stability

The purpose of this study is to evaluate the stability of the specimen after it has been collected in the INDICAID® COVID-19 Antigen Test Buffer Solution. In this study, test samples were tested immediately (< 1 minute), 15, 30, 60, and 120 minutes after collection in the Buffer Solution Vial. Each condition was tested in triplicate using contrived samples of 3x LoD (8.4×10^3 $TCID_{50}$/mL or 4.2×10^2 $TCID_{50}$/swab) inactivated SARS-CoV-2 in pooled healthy human nasal matrix. Negative samples of pooled health human nasal matrix were also evaluated in triplicate for each incubation duration. The results of each test were evaluated after 20 minutes. All replicates from each incubation time produced the expected positive or negative result when a test sample with 3x LoD SARS-CoV-2 or negative pooled nasal matrix, respectively, was applied to the test devices. No invalid test results were observed. After specimen collection, samples of SARS-CoV-2 virus in nasal matrix may be stored in INDICAID® COVID-19 Rapid Antigen Test Buffer Solution Vial for up to 120 minutes at room temperature without impacting product performance.

For detailed testing procedures and raw data, please refer to Appendix K10 and M10.

Cross Reactivity

The purpose of this study is to demonstrate that the INDICAID® COVID-19 Rapid Antigen Test does not react with non-SARS-CoV-2 pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen.

Cross-reactivity of common respiratory pathogens with the INDICAID® COVID-19 Rapid Antigen Test was evaluated by testing the panel of microorganisms at the concentration presented in the table below. Each microorganism was prepared in pooled human nasal matrix from healthy donors in absence of SARS-CoV-2 and tested in triplicate. No cross-reactivity was observed for the following organisms when tested at the concentration listed.

Microbial interference of common respiratory pathogens with the INDICAID® COVID-19 Rapid Antigen Test was also evaluated by testing the panel of microorganisms at the concentration presented in the table below. The microorganisms were tested in a pool of 2 to 4 organisms per pool in the presence of irradiated SARS-CoV-2 (3x LoD, 8.4×10^3 TCID50/mL, or 4.2×10^2 TCID50/swab). Each sample was tested in triplicate. The study was performed whereby if a pooled sample was found to interfere with the test, the individual organisms are tested to identify the highest concentration that does not interfere with the test. No interference was observed for the organisms tested at the concentration listed.

Type	Potential Cross-reactant	Test Concentration
Bacteria	*Bordetella pertussis A639*	1.0 x 10^6 CFU/mL
	Chlamydia Pneumoniae	1.0 x 10^6 IFU/mL
	Haemophilus influenzae	1.0 x 10^6 CFU/mL
	Legionella pneumophila	1.0 x 10^6 CFU/mL
	Mycoplasma pneumoniae	1.0 x 10^6 CFU/mL
	Streptococcus pneumoniae	1.0 x 10^6 CFU/mL
	Streptococcus pyrogenes	1.0 x 10^6 CFU/mL
	Staphylococcus aureus	1.0 x 10^6 CFU/mL
	Staphylococcus epidermidis	1.0 x 10^6 CFU/mL
Virus	Human coronavirus 229E	1.0 x 10^5 $TCID_{50}$/mL
	Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL
	Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL
	Adenovirus	1.0 x 10^5 $TCID_{50}$/mL
	Human Metapneumovirus (hMPV)[1]	1.6 x 10^4 $TCID_{50}$/mL
	Influenza A	1.0 x 10^5 $TCID_{50}$/mL
	Influenza B	1.0 x 10^5 $TCID_{50}$/mL
	Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL
	Parainfluenza Virus Type 1	1.0 x 10^5 $TCID_{50}$/mL
	Parainfluenza Virus Type 2	1.0 x 10^5 $TCID_{50}$/mL
	Parainfluenza Virus Type 3	1.0 x 10^5 $TCID_{50}$/mL
	Parainfluenza Virus Type 4	1.0 x 10^5 $TCID_{50}$/mL
	Enterovirus Type 68	1.0 x 10^5 $TCID_{50}$/mL
	Respiratory Syncytial Virus Type A	1.0 x 10^5 $TCID_{50}$/mL
	Respiratory Syncytial Virus Type B	1.0 x 10^5 $TCID_{50}$/mL
	MERS-Coronavirus	1.0 x 10^5 $TCID_{50}$/mL
	SARS-Coronavirus[2]	Ct = 25-28

[1] Human Metapneumovirus (hMPV) was supplied with a stock concentration lower than the recommended test concentration, and therefore could only be tested at the stock concentration.

[2] Cycle threshold (Ct) range based on supplier's real-time PCR assay targeting the envelope/membrane protein gene region. Due to facility limitations, we were unable to test active SARS-Coronavirus whole virus, a BSL 3 organism. An inactivated SARS-Coronavirus was sought and the only commercially available product that was found is NATrol™ Coronavirus-SARS Stock (Zeptometrix, #NATSARS-ST). This product contains purified, intact virus that has been chemically modified to render them non-infectious. It is supplied in a purified protein matrix that mimics the composition of a true clinical specimen. Its intended use is designed to evaluate the performance of nucleic acid tests but can also be used for validation of clinical assays and diagnostics tests, according to the manufacturer. As such, the concentration is given in terms of Ct values rather than $TCID_{50}$/mL. Undiluted stock was used for testing in this study.

Yeast	Candida albicans	1.0 x 10^6 CFU/mL
Other	Pooled human nasal wash	100%

Therefore, no cross-reactivity or microbial interference is observed for the INDICAID® COVID-19 Rapid Antigen Test with the microorganisms and concentrations that were evaluated in this study.

For detailed testing procedures and raw data, please refer to Appendix K5 and M5.

Endogenous Interference

The purpose of this study is to evaluate how potentially interfering substances may affect the detection of SARS-CoV-2 nucleocapsid antigen by the INDICAID® COVID-19 Rapid Antigen Test. This study investigated whether potentially interfering substances that may be found in the upper respiratory tract in symptomatic patients (including over-the-counter medications) cross-react or interfere with the detection of SARS-CoV-2 by INDICAID® COVID-19 Rapid Antigen Test. 14 endogenous substances were evaluated for potential interference with the INDICAID® COVID-19 Rapid Antigen Test. No interference was observed at the concentrations of the endogenous substances evaluated in the presence of 3x LoD SARS-CoV-2 (8.4 x 10^3 $TCID_{50}$/mL or 4.2 x 10^2 $TCID_{50}$/swab). However, 5% v/v fluticasone propionate showed a tendency reduce the line intensities of the test when compared to the control samples that were absent of any endogenous substances.

For detailed testing procedures and raw data, please refer to Appendix K6 and M6.

Reproducibility

The purpose of this study is to determine if the INDICAID® COVID-19 Rapid Antigen Test produces consistent and accurate test results when performed by different users, using different lots, and on different testing days.

Contrived samples of 3x LoD (8.4 x 10^3 $TCID_{50}$/mL or 4.2 x 10^2 $TCID_{50}$/swab), 1x LoD (2.8 x 10^3 $TCID_{50}$/mL or 1.4 x 10^2 $TCID_{50}$/swab), 1/3x LoD (9.3 x 10^2 $TCID_{50}$/mL or 46.7 $TCID_{50}$/swab), and 0 $TCID_{50}$/mL gamma-irradiated SARS-CoV-2 in pooled nasal matrix, were tested on the INDICAID® COVID-19 Rapid Antigen Test. Testing was performed on three product lots by two different study operators, over three different testing days. On each testing day triplicate tests were performed for each lot of product at each contrived sample level by both operators. Each operator was instructed to interpret the results of his or her own set of tests as well as the results of the other operator's tests.

Test result reproducibility was evaluated by calculating the % agreement between the observed and expected test result for all replicates per testing day, as well as for all replicates tested over the entire study. The result showed that the INDICAID® COVID-19 Rapid Antigen Test satisfies the performance specifications for reproducibility.

For detailed testing procedures and raw data, please refer to Appendix K7 and M7.

Validation Study

The purpose of this study is to establish the clinical validation (positive and negative percent agreement with the natural positive and negative clinical specimens) of the INDICAID COVID-19 Rapid Antigen Test.

50 positive and 50 negative SARS-CoV-2 retrospective NP swab eluate samples, confirmed by FDA EUA RT-PCR SARS-CoV-2 tests (Hologic Panther Fusion SARS-CoV-2 Test or TaqPath COVID-19 Combo Kit), were purchased from Boca Biolistics and Lee Biosciences. The samples were tested with the INDICAID® COVID-19 Rapid Antigen Test by two untrained operators over two non-consecutive days.

On each testing day, a Study Observer randomized and blind-labeled the samples such that the Study Operators did not know the SARS-CoV-2 status. After randomizing the samples, the Study Observer prepared the test samples by inoculating 50 µL of NP swab eluate onto the swab provided in the INDICAID® COVID-19 Rapid Antigen Test. The inoculated swabs were then given to one of two untrained Study Operators to perform the INDICAID® test as instructed in the User Manual. The test results were interpreted and recorded by the Study Operators without assistance from the Study Observer.

INDICAID® COVID-19 Rapid Antigen Test	**Comparator Method (FDA EUA RT-PCR Test)**		
	Positive	**Negative**	**Total**
Positive	48	0	48
Negative	2	50	52
Total	50	50	100
Positive Percent Agreement (PPA)	96% (95% CI: 86.3% - 99.5%)		
Negative Percent Agreement (NPA)	100% (95% CI: 92.9% - 100%)		

The INDICAID® COVID-19 Rapid Antigen Test correctly detected 48 of 50 positive samples and demonstrated no false positives for the negative samples. Based on these data, the INDICAID® test satisfies the performance criteria of 80% positive percent agreement and 80% negative percent agreement with the comparator method.

For detailed testing procedures and raw data, please refer to Appendix K8 and M8.

Point-of-Care Flex Study

The purpose of Point-of-Care (POC) Flex Studies is to assess potential sources of operator error or out-of-specification procedures on the performance or the results of the INDICAID® COVID-19 Antigen Test. This POC Flex Study will evaluate the effect of applying the non-recommended Buffer Solution volume to the Test Device.

Contrived samples of 2x LoD (5.6×10^3 $TCID_{50}$/mL or 2.8×10^2 $TCID_{50}$/swab) gamma-irradiated SARS-CoV-2 in pooled nasal matrix, as well as non-spiked negative pooled nasal matrix, were tested on the INDICAID® COVID-19 Rapid Antigen Test (n=3 per condition). Following the release of contrived specimen from the inoculated swab into the Buffer Solution, solution volumes of 1, 2, 3, 4, 5, 6 drops, and the entire Buffer Solution Vial volume were applied to the Test Device. Test results for all replicates were interpreted at 20 minutes.

Application of 1 drop of Buffer Solution to the Test Device produced invalid test results (no control line present) for all replicates regardless of the presence or absence of SARS-CoV-2 in the contrived sample. Buffer Solution failed to flow through the entire length of the test strip.

All Test Device replicates that received 2-6 drops of Buffer Solution produced the expected positive or negative result when a test sample with 2x LoD SARS-CoV-2 or negative pooled nasal matrix, respectively, was applied.

Test Devices that received the full Buffer Solution volume produced 2 of 3 false negative test results for samples containing 2x LoD SARS-CoV-2. All three replicates that received full Buffer Solution without SARS-CoV-2 produced the expected negative results. Applying the full Buffer Solution volume led to leakage of solution around the edges of the Test Device cassette.

In conclusion, with low positive SARS-CoV-2 samples (i.e. 2x LoD), accurate test results are produced when 2-6 drops from the Buffer Solution Vial are applied to the INDICAID® COVID-19 Rapid Antigen Test. However, it is recommended that 3 drops of sample in Buffer Solution are applied to the test for the most consistent and accurate results.

For detailed testing procedures and raw data, please refer to Appendix K9 and M9.

Read Time Variability Study

The purpose of Point-of-Care (POC) Flex Studies is to assess potential sources of operator error or out-of-specification procedures on the performance or the results of the INDICAID® COVID-19 Antigen Test. This POC Flex Study evaluates the effect of reading the test results at various times before and after the recommended read time stated in the User Manual.

Test results were read at times of 5, 10, 15, 20 (recommended read time), 30, and 60 minutes after sample in Buffer Solution was applied to the Test Device. Each read time condition was evaluated in triplicate using contrived samples of 2xLoD (5.6×10^3 $TCID_{50}$/mL or 2.8×10^2 $TCID_{50}$/swab) gamma-irradiated SARS-CoV-2 in pooled human nasal matrix. Negative samples of pooled human nasal matrix were also evaluated in triplicate at each time point. The result shows that with low positive SARS-CoV-2 samples (i.e. 2x LoD), accurate test result interpretation can be made as soon as 10 minutes and as high as 60 minutes after samples have been applied to the INDICAID® COVID-19 Rapid Antigen Test. However, it is recommended that test results are interpreted at 20 minutes. For detailed testing procedures and raw data, please refer to Appendix K11 and M11.

High Temperature and Humidity Study

The purpose of Point-of-Care (POC) Flex Studies is to assess potential sources of operator error or out-of-specification procedures on the performance or the results of the INDICAID® COVID-19 Antigen Test. Contrived samples of 2x LoD (5.6×10^3 $TCID_{50}$/mL or 2.8×10^2 $TCID_{50}$/swab) gamma-irradiated SARS-CoV-2 in pooled nasal matrix, as well as non-spiked negative pooled nasal matrix, were tested on the INDICAID® COVID-19 Rapid Antigen Test (n=3 per condition). One hour prior to running the study, test kits were placed in an incubator capable of maintaining a temperature near 40°C and relative humidity near 95%. Contrived samples were then applied to the Test Devices and then allowed to run for 20 minutes in the same high temperature/humidity incubator. Study operators interpreted and recorded the test results after the 20-minute run time. With low positive SARS-CoV-2 samples (i.e. 2x LoD), accurate test results are still produced when the INDICAID® COVID-19 Rapid Antigen Test is performed in high temperature and high relative humidity climates (i.e. ~40°C / ~95% RH). All Test Device replicates produced the expected positive or negative result when a test sample with 2x LoD SARS-CoV-2 or negative pooled nasal matrix, respectively, was applied, regardless of the temperature/humidity condition. No invalid test results were observed.

For detailed testing procedures and raw data, please refer to Appendix K12 and M12.

Low Temperature Study

The purpose of Point-of-Care (POC) Flex Studies is to assess potential sources of operator error or out-of-specification procedures on the performance or the results of the INDICAID® COVID-19 Antigen Test. This POC Flex Study evaluates the effect of performing the test at low temperature to mimic cold climates.

The performance of the INDICAID® COVID-19 Rapid Antigen Test, when performed in conditions that mimic cold climates, will be assessed by placing test kits in a refrigerator capable of maintaining a temperature of 2-8°C. Kits placed at room temperature (15-30°C) and RH (30-50%) of the controlled laboratory environment will serve as test controls. Each condition will be tested in triplicate with test samples containing 2xLoD gamma-irradiated SARS-CoV-2 in negative pooled human nasal matrix, or with test samples of negative pooled human nasal matrix only.

For detailed testing procedures and raw data, please refer to Appendix K14 and M13.

3.9 Stability Testing and Shelf life

Product Shelf life

The objective of this stability study protocol is to verify the INDICAID® COVID-19 Rapid Antigen Test device shelf life claim at the intended storage condition as part of the design verification activity. Accelerated stability study will be used initially to predict the shelf life of the device. A real-time stability study will be performed concurrently to confirm the accelerated study results and to confirm the product's shelf life claim. The study will test three (3) different kit lots for both the accelerated and real-time stability studies.

Three concentration levels of inactivated SARS-CoV-2 (3 x LoD, 1 x LoD & 1/3 x LoD) diluted in pooled human nasal matrix obtained from healthy donors will be tested with five replicates per level. Non-spiked negative nasal matrix will also be tested with five replicates. 300 tests (100 tests per lot) will be performed in each Accelerated stability study and Real-time stability study.

An accelerated shelf-life study was performed at a stress temperature of 50°C for 49 days, and the Arrhenius Equation was used to predict the shelf-life of the INDICAID® COVID-19 Rapid Antigen Test. Assuming an ambient (real-time) temperature of 30°C, the shelf-life is predicted to be 12 months. A real-time study is on-going to confirm the actual shelf-life of the test.

For detailed testing procedures and raw data, please refer to Appendix K3 and M3.

Shipping Stability

The purpose of this study is to assess how shipping conditions may impact the INDICAID® COVID-19 Rapid Antigen Test Kit packaging integrity and/or product

performance. One lot of tests was used for this study. Final kits contained the supplied items listed on the User Manual were subjected to simulated shipping temperature study and shipping evaluation.

In the simulated shipping temperature study, the INDICAID® COVID-19 Rapid Antigen Test Devices, Buffer Solution Vials, and collection swabs were subjected to temperature conditions of -15 °C ± 5°C, 2°C – 8°C, room temperature (15°C – 30°C), or 50°C ± 5°C for three days. At the end of the three-day incubation, product performance was evaluated using contrived test samples of 3x LoD SAR-CoV-2 virus (8.4×10^3 $TCID_{50}$/mL or 4.2×10^2 $TCID_{50}$/swab) in 1:200 pooled human nasal fluid/1xPBS. 1:200 pooled human nasal fluid/1xPBS without SARS-CoV-2 was also tested as the negative clinical matrix control. All product materials were brought to room temperature prior to testing and each condition was tested in triplicate. The result showed that three days exposure of the INDICAID® COVID-19 Rapid Antigen Test to temperature conditions that may be encountered during shipping/transporting of the product does not appreciably impact product performance.

In the shipping evaluation study, two INDICAID® COVID-19 Rapid Antigen Test Kits (25 tests per kit) composed of final product materials and packaging were shipped from Phase Diagnostics (Garden Grove, CA) to a designated site in Hong Kong, followed by return shipment to Phase Diagnostics. During the return shipment, the test kits were held for U.S. customs inspection for 18 days before final delivery. Post-shipment inspection of both test kits was performed by a Phase Diagnostics employee to visually check the product in terms of box integrity, label integrity and legibility, tube leakage/breakage, and integrity of the box contents. The real shipping evaluation demonstrated that the integrity of product packaging, labels, and seals is maintained.

For detailed testing procedures and raw data, please refer to Appendix K4 and M4.

3.10 Specification of Materials and Processes

- Appendix L1 – RMS-0181 INDICAID COVID-19 Rapid Antigen Test Pouched Cassette Device
- Appendix L2 – RMS-0180 INDICAID COVID-19 Rapid Antigen Test Buffer Solution Vial
- Appendix L3 – RMS-0157 Disposable Sampler Swab
- Appendix L4 – RMS-0171 INDICAID COVID-19 Rapid Antigen Test Kit Box – 25 test kit
- Appendix L5 – RMS-0196 INDICAID COVID-19 Rapid Antigen Test Kit Box – 5 test kit
- Appendix L1 – RMS-0183 INDICAID COVID-19 Rapid Antigen Test Kit Box – 1 test kit

3.11 Flow of Manufacturing Process

a. Kit Production procedure (Please refer to Appendix P for the detail Production Workflow)

i) Sekbio produce bulk package of Buffer Solution and whole piece of LFA paper card. Then Sekbio will send the raw material to SZPSI.
ii) SZPSI re-label all raw materials, then send to Dispensing OEM and Cassette OEM, respectively.
iii) Dispensing OEM dispend the solution into individual bottles and sticking label on the bottles. After processing, Dispensing OEM will send the materials to HKPSI.
iv) Cassette OEM cut the whole LFA paper card into strips and pack those into individual cassette, then they will print label information onto the package. After processing, Cassette OEM will send the materials to HKPSI.
v) HKPSI pack required number of separate vials into bags and put label stickers onto kit boxes. After processing, the components will be assembled into kits as instructed. Kit will be sealed with clear round label.

3.12 Literature

Literatures	Source	Appendix	Selection justification
Lateral flow assays	Essays in Biochemistry	Q1	It provides a proof of concept of the Lateral flow assays (LFAs) are the technology behind low-cost, simple, rapid and portable detection devices and overview of the principle of the LFAs method.
Detection of COVID-19: A review of the current literature and future perspectives	Biosensors and Bioelectronics	Q2	It shows that detection of the NP antigen might be an effective strategy for the early screening of suspected SARS-CoV or MERS-CoV-infected patients
Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein	medRxiv	Q3	It provides a justification that nucleocapsid protein assay is an accurate, rapid, early and simple method for diagnosis of COVID-19
Nasal Swab Sampling for SARS-CoV-2: a Convenient Alternative in Times of Nasopharyngeal Swab Shortage	Journal of Clinical Microbiology	Q4	It provides a justification that the molecular detection of SARS-CoV-2 using nasal swab specimens was nearly equivalent to the detection using nasopharyngeal swab considered the gold standard.
Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection (Review)	Cochrane Database of Systematic Reviews	Q5	It reviewed that antigen test varied considerably across studies but with high average specificity.

Evaluation of rapid antigen test for detection of SARS-CoV-2 virus	Journal of Clinical Virology	Q6	It suggests that rapid antigen detection test device may be a useful mass screening test when RT-PCR assays are not or insufficiently available, in particular in symptomatic patients.

3.13 Post Market Surveillance

Refer to Appendix N for SOP-0021 Customer Complaints Procedure

3.14 Revision History and Approval

Revision History	Approval information
New Document, Rev. A	CO-######-##