WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: Sure Status COVID-19 Antigen Card Test
Manufacturer: Premier Medical Corporation Private Limited
EUL Number: EUL 0590-010-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and the Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: a desktop review of the manufacturer's Quality Management System documentation and specific manufacturing documents.
- Product Dossier Review assessment of the documentary evidence of safety and performance. This evaluation of limited scope is to verify critical analytical and performance characteristics.

Sure Status COVID-19 Antigen Card Test, product codes SS03P25 and SS03-NS-P25, Rest of World regulatory version, manufactured by Premier Medical Corporation Private Limited, A1-302, GIDC Sarigam, District, Valsad 396155, India, was listed on 17 March 2021.

Report amendments and product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Addition of nasopharyngeal swabs not included in the product dossier reviewed before listing—inclusion of labels of approved nasopharyngeal swabs in the public report.	4 August 2022
3.0	Introduction of a new product code SS03-NS-P25 that uses nasal swab specimen type and changes to the sample pad to filter the sticky part of the nasal discharge.	8 November 2022

Intended use:

According to the claim of intended use from Premier Medical Corporation Private Limited, "*Sure Status COVID-19 Antigen Card Test is a lateral flow immunochromatographic assay for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal (NP) and nasal swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider.*
Sure Status COVID-19 Antigen Card Test is for in vitro diagnostic use and intended as an aid to the detection of nucleocapsid protein antigen in a patient with clinical symptoms of SARS-CoV-2 infection. It provides only initial screening test results and a more specific alternative diagnosis method should be performed to obtain the confirmation of SARS-CoV-2 infections. The test is not automated and does not require any additional instrument. The test is designed to be performed by Laboratory professionals/trained users only. The product can be used in a clinical setup and point of care sites that meets the requirements stated in these instructions for use or local regulations."

Validated specimen type
Nasopharyngeal swab specimens.

Test kit contents

Component	**25 tests (product code SS03P25)**	**25 tests (product code SS03-NS-P25)**
Test device pouch containing: test device and desiccant	25	25
Swab	25 (Nasopharyngeal swabs)	25 (Nasal swabs)
Reaction buffer vial with nozzle	25	25
Extraction Buffer Bottle	3 x 3 mL	5 x 3 mL
Instructions for use	1	1
External Positive Control Swab	\	1
External Negative Control Swab	\	1

Items required but not provided

- New pair of disposable gloves & facemask;
- Permanent marker pen and Timer;
- PPE Kits;
- Biohazardous waste container.

Storage
4-30°C.

Shelf-life upon manufacture
24 months (real-time stability studies are ongoing).

Warnings/limitations
Refer to the instructions for use (IFU).

Product dossier assessment

Premier Medical Corporation Private Limited submitted a product dossier for the Sure Status COVID-19 Antigen Card Test for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid and rapid diagnostics tests detecting SARS-CoV-2 antigens (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, Premier Medical Corporation Private Limited committed to,

1. Participate in the WHO collaborative study to assess the suitability of an interim standard for SARS-CoV-2 virus antigen detection tests.
2. When it becomes available, submit a study report on the estimation of the analytical sensitivity with the WHO International Standard.
3. When this becomes available, submit a study report on the traceability of all relevant materials to the International SARS-CoV-2 virus standard.
4. To submit interim and final stability reports by 30 June 2023.

Risk-benefit assessment conclusion is acceptable.

Quality Management Systems Review

To establish eligibility for WHO procurement, Premier Medical Corporation Private Limited was asked to provide up-to-date information about the status of its quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that Premier Medical Corporation Private Limited provided sufficient information to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid or antigen (PQDx_347)*".

Quality management system assessment outcome: Acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:
1. Notification to WHO of any planned changes to a EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9).

Premier Medical Corporation Private Limited is also required to report complaints related to the product. Specific categories of complaints and changes to the product must be notified immediately to WHO, as per the documents mentioned above.

The manufacturer has committed to ensuring that post-emergency use listing safety, quality and performance monitoring activities are in accordance with WHO guidance "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics*" (ISBN 978-92-4-001531-9).[1]

Scope and duration of procurement eligibility

Sure Status COVID-19 Antigen Card Test, product codes SS03P25 and SS03-NS-P25, manufactured by Premier Medical Corporation Private Limited, is considered eligible for WHO procurement until further notice. The assay may detect the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antigen. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO-prequalified.

As part of the ongoing requirements for listing as eligible for WHO procurement, Premier Medical Corporation Private Limited must engage in post-market surveillance activities to ensure that the product meets safety, quality and performance requirements. Premier Medical Corporation Private Limited must notify WHO of any complaints, including adverse events related to the use of the product, within seven days and any changes to the product.

WHO reserves the right to rescind eligibility for WHO procurement if additional information on the safety, quality, and performance during post-market surveillance activities and if new data becomes available to WHO that changes the risk-benefit balance.

[1] Available on the web page https://www.who.int/publications/i/item/guidance-for-post-market-surveillance-and-market-surveillance-of-medical-devices-including-in-vitro-diagnostics

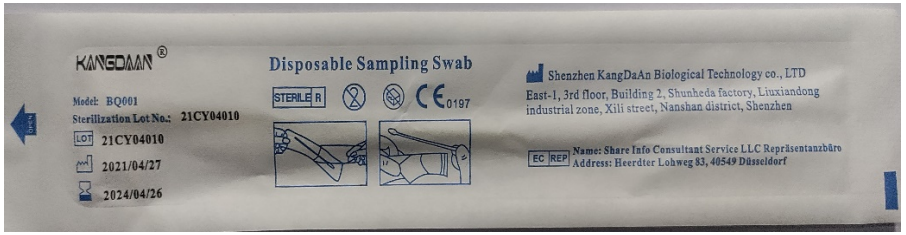
Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

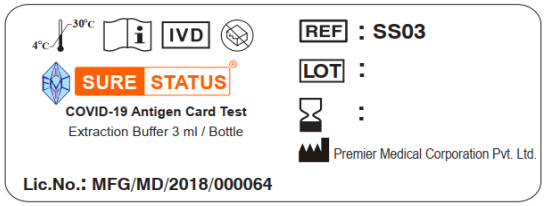
1.0 Product labels

1.01) Nasopharyngeal Swab Labels

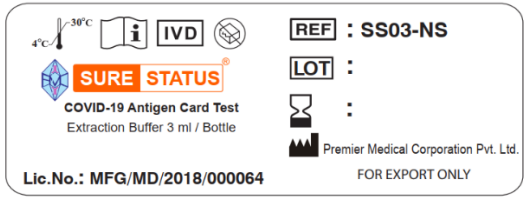


1.02) Nasal Swab Labels

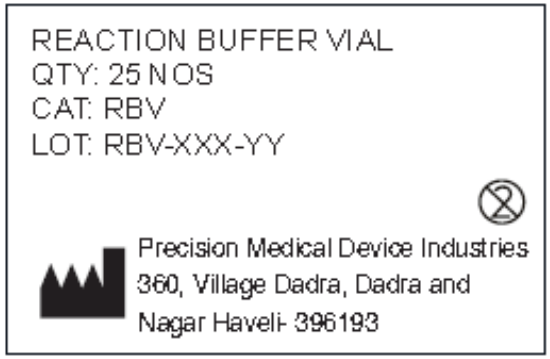
1.03) Extraction Buffer Bottle Label for SS03P25



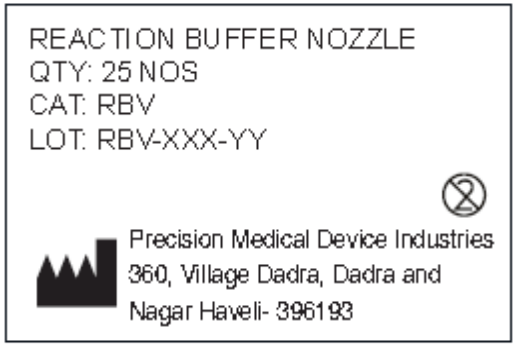
1.04) Extraction Buffer Bottle Label for SS03-NS-P25



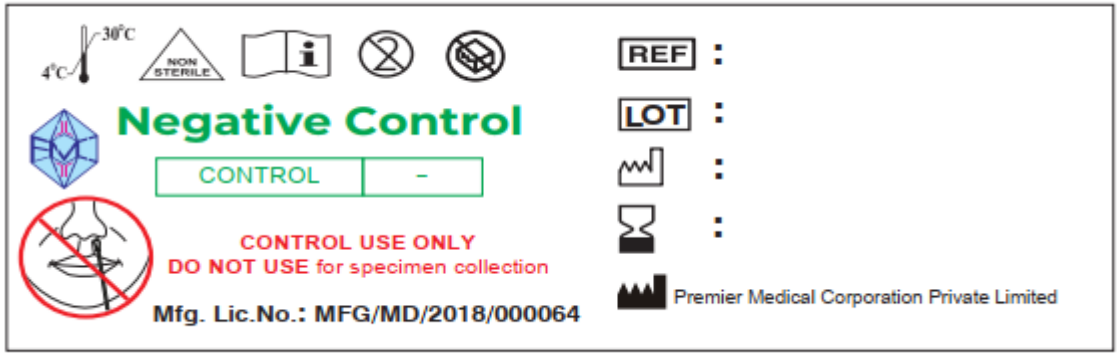
1.05) Reaction buffer nozzle bag label



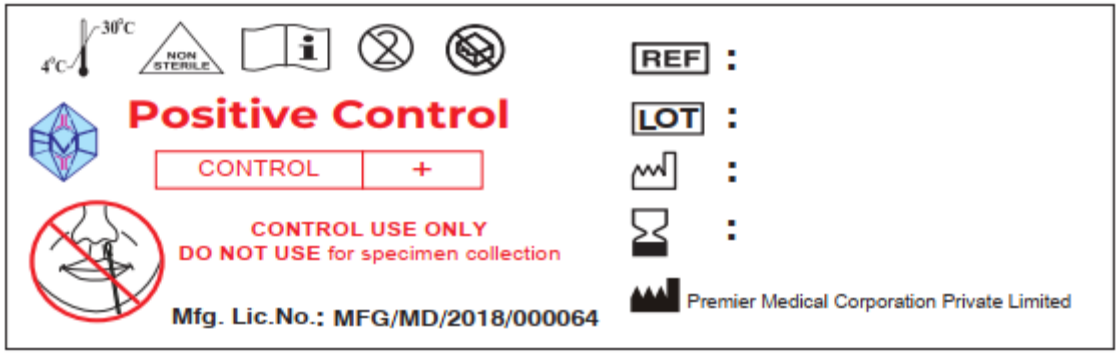
1.06) Reaction buffer vial bag label



1.07) Negative Control Swab Label for SS03-NS-P25

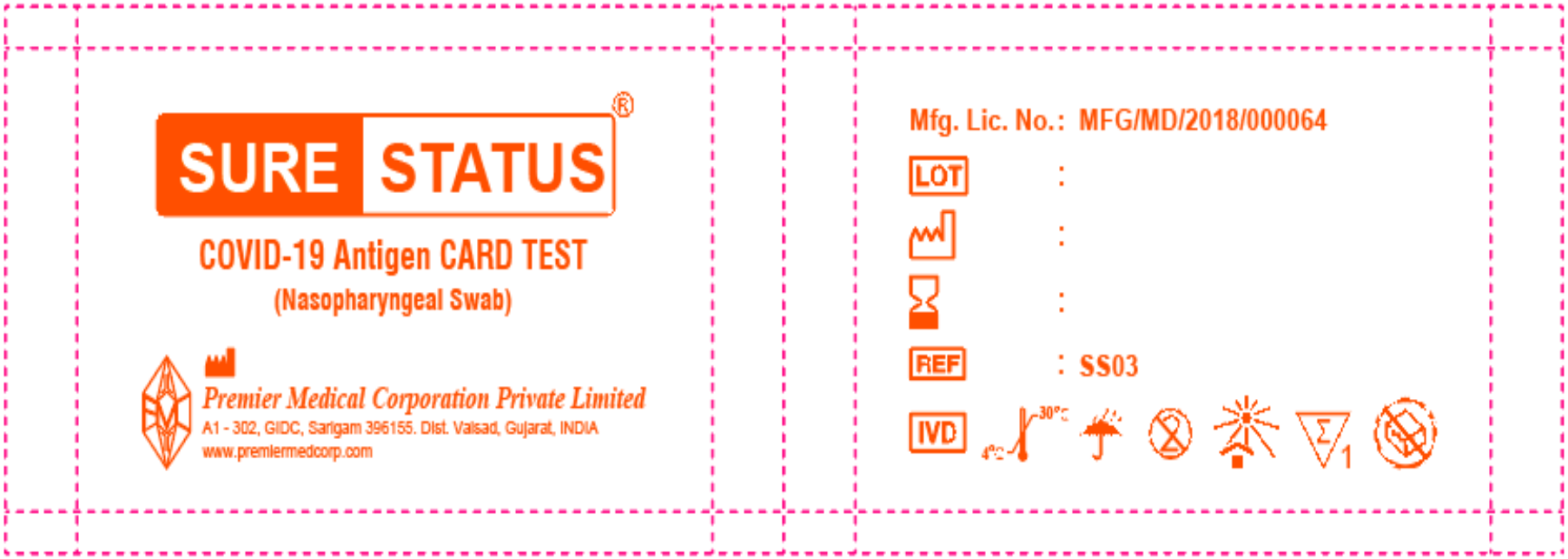


1.08) Positive Control Swab Label for SS03-NS-P25

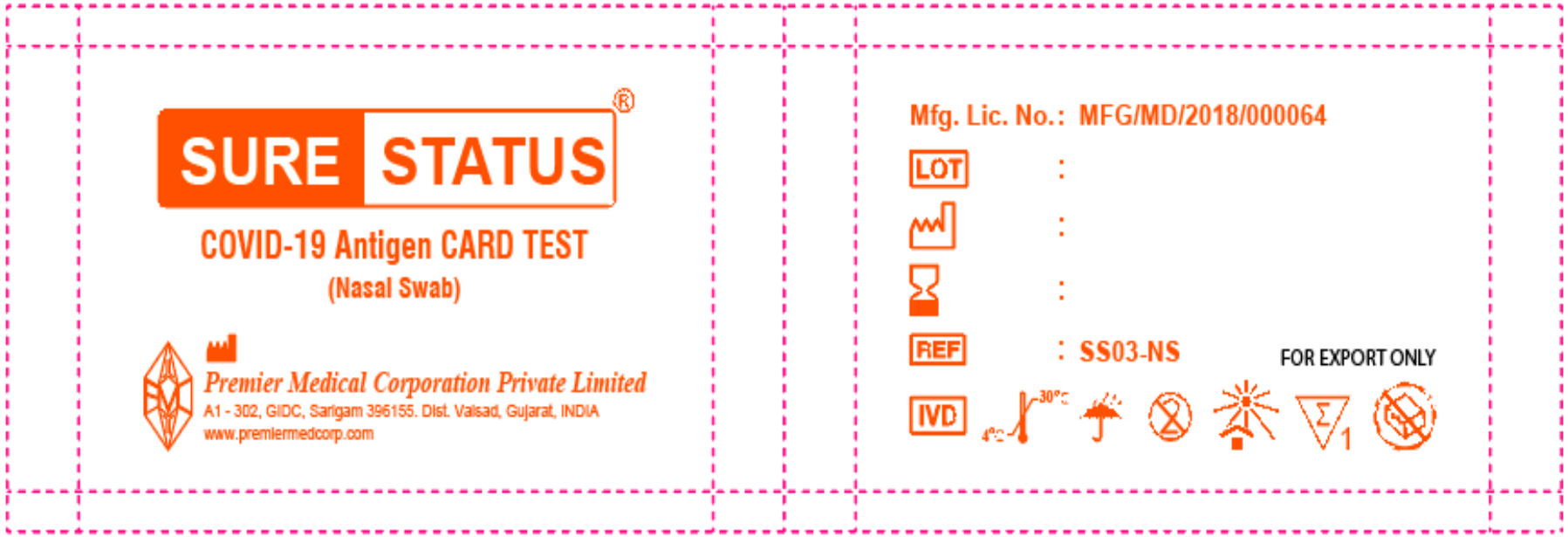


1.09) Test Device Pouch

a) Test Device Pouch label for SS03P25



b) Test Device Pouch label for SS03-NS-P25



1.10) Test device

1.11) Outer Box Label

a) Carton label for SS03P25

Mfg. Lic. No. : MFG/MD/2018/000064

LOT :

:

:

Contents:

• Individually pouched test devices with desiccant	: 25 Nos.
• Nasopharyngeal swab	: 25 Nos.
• Reaction buffer vial with nozzle	: 25 Nos.
• Extraction Buffer Bottle	: 3 Nos.
• Instructions for use	: 1 No.

Rev. A0, 2021-08

REF SS03P25

25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS

COVID-19 ANTIGEN CARD TEST

(Nasopharyngeal Swab)

4°C-30°C

IVD

For Professional Use.

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155, Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF SS03P25

25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS

COVID-19 ANTIGEN CARD TEST

(Nasopharyngeal Swab)

REF SS03P25

25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS

COVID-19 ANTIGEN CARD TEST

(Nasopharyngeal Swab)

For *in vitro* Diagnostic Use Only.

b) Carton label for SS03-NS-P25

Mfg. Lic. No.: MFG/MD/2018/000064

LOT :

:

:

Contents:

• Individually pouched test devices with desiccant	: 25 Nos.
• Nasal swab	: 25 Nos.
• Reaction buffer vial with nozzle	: 25 Nos.
• Extraction Buffer Bottle	: 5 Nos.
• Positive control swab	: 1 No.
• Negative control swab	: 1 No.
• Instructions for use	: 1 No.

Rev.: Draft

REF SS03-NS-P25
25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS®

COVID-19 ANTIGEN CARD TEST
(Nasal Swab)

FOR EXPORT ONLY
For in vitro Diagnostic Use Only.

REF SS03-NS-P25
25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS®

COVID-19 ANTIGEN CARD TEST
(Nasal Swab)

4°C-30°C IVD

For Professional Use.

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REF SS03-NS-P25
25 Tests/kit

Advanced Quality Screening of COVID-19 Antigen

4°C-30°C

SURE STATUS®

COVID-19 ANTIGEN CARD TEST
(Nasal Swab)

3.0 Instructions for use[2]

[2] English version of the IFU was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

a) Instructions for Use of SS03P25

ENDOGENOUS INTERFERING SUBSTANCE STUDIES

A study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract in symptomatic subjects (including over the counter medications) do not cross- react or interfere with the detection of SARS-CoV-2 in the Sure Status® COVID-19 Antigen Test. Each substance was tested in triplicate in the absence or presence of SARS-CoV-2 at 67.2-70 $TCID_{50}$/swab (3X LOD).

No interference was seen with the following endogenous interfering substances when tested at the concentration presented in the table below.

Sr. No	Interfering Substance	Active Ingredient	Concentration
1	Endogenous	Mucin	2% w/v
2		Blood (human)	5% v/v
3	Nasal Gel	Sodium Chloride (NeilMed)	5% v/v
4	Nasal Drops	Phenylephrine	15% v/v
5	Nasal spray	Cromolyn	15% v/v
6		Oxymetazoline	15% v/v
7		Fluconazole	5% v/v
8	Homeopathic Nasal Spray	Alkalol	10% v/v
9		Fluticasone Propionate	0.5% v/v
10		Zincum gluconium (Zicam)	5% v/v
11	Sore Throat Phenol Spray	Phenol	15% v/v
12	Throat Lozenge	Benzocaine	0.15% v/v
13		Menthol	0.15 % v/v
14	Antiviral Drug	Zanamivir	300 ng/ml
15		Oseltamivir Phosphate (Tamiflu)	0.5% v/v
16	Antibiotic, systemic	Tobramycin	0.0004 % w/v
17	Antibiotic, Nasal ointment	Mupirocin	0.25 w/v
18	Allergy medication	Diphenhydramine (Benadryl)	0.1 % w/v
19	Anti- inflammatory medication	Dexamethasone	0.1 % w/v
20	Other substances#	Biotin	100 µg/ml
21		HAMA	372 ng/ml
22		HAMA	297.2 ng/ml
23		Rheumatoid factor	4200 IU/ml

#Note: Other substances (Biotin, HAMA, and Rheumatoid factor) were tested with 70 $TCID_{50}$/swab specimens which are 3XLOD of a specimen.

CROSS-REACTIVITY AND MICROBIAL INTERFERENCE STUDY (ANALYTICAL SPECIFICITY)

Cross-reactivity of the Sure status® COVID-19 Antigen Card Test was evaluated by testing a panel of related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen and could potentially cross-react with the Sure Status® COVID-19 Antigen test including various microorganisms, viruses, and negative matrix.

Cross-Reactivity and potential interference of Sure status® COVID-19 Antigen Card Test was evaluated by testing sixteen (16) viruses, thirteen (13) micro-organisms and two (2) negative matrices. Each organism and virus were tested in triplicate in the absence or presence of gamma irradiated SARS-CoV-2 at 3 X the concentration of LOD (67.2 $TCID_{50}$/swab). The final concentration of the organisms and viruses used in the study are documented in the table below (10^6 CFU/mL or higher for bacteria and 10^5 PFU/mL or higher for viruses was studied).

No cross-reactivity or interference was seen with the following microorganisms when tested at the concentration presented in the table below

Sr. No		Potential Cross-Reactant	Concentration
1	Virus	MERS-CoV EMC/2012 (gamma-irradiated)	8.9 X 10^5 $TCID_{50}$/ml
2		Human coronavirus 229E	1 x 10^5 $TCID_{50}$/ml
3		Human coronavirus OC43	1 x 10^5 $TCID_{50}$/ml
4		Human coronavirus NL63	1.6 x 10^5 $TCID_{50}$/ml
5		Adenovirus, Type-07 (Species B)	1.0 x 10^5 PFU/mL
6		Human Metapneumovirus (hMPV)	1.0 x 10^5 $TCID_{50}$/ml
7		Parainfluenza virus Type 1	1.0 x 10^5 $TCID_{50}$/ml
8		Parainfluenza virus Type 2	1.0 x 10^5 $TCID_{50}$/ml
9		Parainfluenza virus Type 3	1.0 x 10^5 $TCID_{50}$/ml
10		Parainfluenza virus Type 4a	1.0 x 10^5 $TCID_{50}$/ml
11		Influenza A H3N2 (Wisconsin/67/05)	1.0 x 10^5 PFU/mL
12		Influenza A H1N1 (A/Brisbane/59/2007)	1.0 x 10^5 PFU/mL
13		Influenza B (Malaysia/2506/04)	1.0 x 10^5 PFU/mL
14		Enterovirus	1.0 x 10^5 PFU/mL
15		Respiratory syncytial virus Type A (RSV-A)	1.0 x 10^5 U/mL
16		Rhinovirus Type 1A	1.0 x 10^5 PFU/mL
17	Bacteria	*Haemophilus influenzae*	1.0 x 10^6 cells/mL
18		*Streptococcus pneumoniae*	1.0 x 10^6 CFU/mL
19		*Streptococcus pyogenes*	1.0 x 10^6 CFU/mL
20		*Bordetella pertussis*	1.0 x 10^6 cells/mL
21		*Mycoplasma pneumoniae*	1.0 x 10^6 CFU/mL
22		*Chlamydia pneumoniae*	1.0 x 10^6 IFU/mL
23		*Legionella pneumophila*	1.0 x 10^6 CFU/mL
24		*Mycobacterium tuberculosis*	1.0 x 10^6 CFU/mL
25		*Pneumocystis jirovecii*	1.0 x 10^6 CFU/mL
26		*Pseudomonas aeruginosa*	1.0 x 10^6 CFU/mL
27		*Staphylococcus epidermidis*	1.0 x 10^6 CFU/mL
28		*Streptococcus salivarius*	1.0 x 10^6 CFU/mL
29	Yeast	*Candida albicans*	1.0 x 10^6 cells/mL
30	Negative matrix	Universal Viral Transport Media	NA
31		Pooled human nasal wash collected for microbial flora	NA

LIMITATIONS

1) Negative results, should be treated as presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed.
2) Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
3) If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
4) Collected specimens may be frozen at -20°C and used up to 10 days after freezing and it is stable for 1 hour in extraction buffer at room temperature (15-30°C).
5) Results from antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
6) This test will indicate the presence of SARS-CoV-2 nucleocapsid protein antigen in the specimen from both viable and non-viable SARS-CoV-2 virus.
7) Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
8) The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
9) Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
10) This device has been evaluated for use with human specimen material only.
11) False-negative results may occur if the concentration of the target antigen in the clinical specimen is below the detection limits of the device.
12) This device is a qualitative test and does not provide information on the viral concentration present in the specimen.
13) This test cannot rule out diseases caused by other bacterial or viral pathogens.
14) Test may give cross reactivity with other strain like Human coronavirus HKU1 or SARS-coronavirus (SARS-CoV-1).
15) False-negative results may occur due to high concentration of analyte (Hook Effect).

REFERENCES

1) https://covid19.who.int
2) https://www.who.int/publications/m/item/covid-19-public-health-emergency-of-international-concern-(pheic)-global-research-and-innovation-forum.

SYMBOL LEGENDS

Symbol	Explanation of symbol
	Consult instructions for use
	Non Sterile
IVD	In vitro diagnostic medical device
	Store at 4-30 °C
	Caution
	Keep dry
	Do not reuse

Symbol	Explanation of symbol
	Contains sufficient for < n > tests
REF	Product Code
LOT	Lot Number
	Manufacturer
	Date of manufacture (YYYY-MM)
	Expiration Date (YYYY-MM)

PRODUCT DISCLAIMER & WARNINGS

Every warnings and precaution should be taken into consideration before using the test. Failure to consider "Precaution, Warning, and Limitations" may not ensure the diagnostic ability and accuracy of this product. The test result may accordingly be affected by environmental factors and/or user error outside of the control of the Manufacturer and Distributor.

A definitive clinical diagnosis should not be based on the results of a single test, but it should be made by the physician after all clinical and laboratory findings have been evaluated.

"In no event shall our company or its distributor is liable for any direct, indirect, punitive, unforeseen, incidental, special consequential damages, to property or life, whatsoever arising out of or connected with an incorrect diagnosis, whether positive or negative, in the use or misuse of this product".

In the event of performance changes or product malfunction, please contact manufacturer.

INSTRUCTIONS FOR USE

Follow the WHO/US CDC Universal Precautions for the safety against Novel Corona Virus (SARS-CoV-2).

Manufactured by

Premier Medical Corporation Private Limited

A1-302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA.

Customer support E-mail : info@premiermedcorp.com

Tel.: +91 2602780112/113 • Website : www.premiermedcorp.com

• ISO 13485 & EN ISO 13485 Certified Company

Part No.: SS03-INS-001, Rev.:AC Date:2021-03-19

ENGLISH

Note : Instructions for use will be printed in local language of the country using the test, if required.

SURE STATUS®

COVID-19 Antigen Card Test

Rapid Immunochromatographic Card Test for the detection of SARS-CoV-2 Antigen in Human Nasopharyngeal swab.

REF SS03P25

IVD 4°C - 30°C

INTENDED USE

Sure Status® COVID-19 Antigen Card Test is a lateral flow immunochromatographic assay for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in nasopharyngeal (NP) swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider.

Sure Status® COVID-19 Antigen Card Test is for in vitro diagnostic use and intended as an aid to the detection of nucleocapsid protein antigen in a patient with clinical symptoms of SARS-CoV-2 infection. It provides only initial screening test results and a more specific alternative diagnosis method should be performed to obtain the confirmation of SARS-CoV-2 infections. The test is not automated and does not require any additional instrument. The test is designed to be performed by Laboratory professionals/trained users only. The product can be used in a clinical setup and point of care sites that meets the requirements stated in these instructions for use or local regulations.

INTRODUCTION

The novel coronavirus 2019-nCoV has recently emerged as a human pathogen in the city of Wuhan in China's Hubei province, causing fever, severe respiratory illness, and pneumonia—a disease recently named COVID-19. According to the World Health Organization (WHO), as of 15 March 2021, there have been 119,960,700 confirmed cases of COVID-19, including 2,656,822 deaths, reported to $WHO_{[1]}$. The emerging pathogen was rapidly characterized as a new member of the betacoronavirus genus, closely related to several bat coronaviruses and severe acute respiratory syndrome coronavirus (SARS-CoV). Compared with SARS-CoV, 2019-nCoV appears to be more readily transmitted from human to human, spreading to multiple continents and leading to the WHO's declaration of a Public Health Emergency of International Concern (PHEIC) on 30 January $2020_{[2]}$. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more severe cases infection can cause pneumonia, severe acute respiratory syndrome (SARS), kidney failure and even death.

Coronavirus can be extracted through respiratory secretions or transmitted through oral fluid, sneezing, physical contact, and other air droplets. There are 2 main proteins involved and necessary for the infection of humans by a coronavirus. Which includes Spike protein (which is a trimeric protein containing 3 different sub-units, among three subunits subunit S2 is highly conserved and subunit S1 is essential to make an entry into host cell) and nucleocapsid protein which is essential for viral assembly.

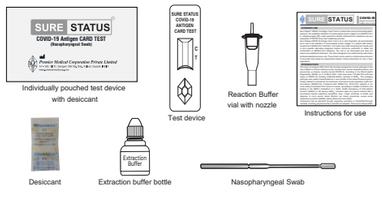
Thus these, 2 proteins play an important role in the infection and viral life cycle in human beings and are the potential target molecules for the development of a rapid test for the detection of coronavirus infection in humans. Sure Status® COVID-19 Antigen Card Test uses nucleocapsid protein as a measurand and particularly, used for the identification of subclinical or asymptomatic cases. It also plays a role in reducing or preventing the transmission of infection as these individuals may transmit the virus. As a Rapid Diagnostic Test with a 15-20 min testing time, Sure Status® COVID-19 Antigen Card Test could be effectively used at a large scale during contact tracing (either detect asymptomatic contacts of a confirmed case or symptomatic acute infection).

ASSAY PRINCIPLE

The detection kit uses the principle of immunochromatography: separation of components in a mixture through a medium using capillary force and the specific and rapid binding of an antibody to its antigen. Each cassette is a dry medium that has been coated separately with anti-coronavirus (anti Nucleocapsid) antibody molecule, this is a monoclonal antibody directed against nucleocapsid protein of 2019-nCoV and goat anti-chicken IgY antibody (control line). 2 free colloidal gold labeled antibodies, i.e anti-nucleocapsid antibody as well as chicken IgY, will be sprayed on the conjugate pad. Once nasopharyngeal swab specimen is collected and diluted in extraction buffer, this diluted specimen will be applied on sample pad. The specimen in buffer will pass through the conjugate pad and bind with anti-nucleocapsid antibody conjugated with colloidal gold on the conjugate pad and will form a complex of antigen-antibody colloidal gold and will migrate towards test and control lines. Thus, the formed complex of antigen-antibody-colloidal gold will migrate through capillary action and binds with the coated antibody molecules at the test line, thus providing a reactive result.

If there is no formation of antigen-antibody colloidal gold complex, it will not bind with the test line and there will not be any development of test lines. Chicken IgY conjugated with colloidal gold will bind with control line antibody irrespective of reactive/non-reactive specimens.

MATERIALS PROVIDED



MATERIALS PROVIDED	PACK SIZE : 25 TESTS
Test device pouch containing: test device and desiccant	25 Nos.
Nasopharyngeal swab	25 Nos.
Reaction buffer vial with nozzle	25 Nos.
Extraction Buffer Bottle (3 ml)	3 Nos.
Instructions for use	1 No.

MATERIALS REQUIRED BUT NOT PROVIDED

- New pair of disposable gloves & facemask
- Permanent marker pen and Timer
- PPE Kits
- Biohazardous waste container

STORAGE AND STABILITY

1) Sure Status® COVID-19 Antigen Card Test kit should be stored at 4-30°C.
2) Do not freeze the kit or components.
3) The kit is sensitive to humidity and heat. Do not store the kit at temperatures above 30°C and in humid conditions.
4) Extraction Buffer Bottle (opened & unopened) & the unopened test device are stable until the expiry date printed on the label, when stored at 4-30°C.
5) Perform the test immediately (within 15 minutes) after removing the test device from the aluminium pouch. If a desiccant color has changed from orange to green, do not use the test device.

PRECAUTIONS

1) Wear protective clothing, masks, gloves and take other appropriate safety precautions to avoid or reduce the risk of infection while handling specimens.
2) Dispose of used gloves as biohazard waste. Wash hands thoroughly afterward.
3) Avoid splashing or aerosol formation.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Decontaminate and dispose of all used specimens, test devices, nasopharyngeal swab, Extraction buffer bottle and Reaction buffer vial with nozzle, in a biohazardous waste container.
6) Sure Status® COVID-19 Antigen Card Test kit requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.

WARNINGS

1) For in vitro diagnostic use only.
2) The test has been authorized only for the presence of specific antigen to SARS-CoV-2 and not for any other viruses or pathogens.
3) Read the instructions carefully before performing the test, any deviation will invalidate the test results.
4) Apply standard biosafety precautions for handling and disposal of potentially infective materials including human biological specimens irrespective of disease state.
5) Do not drink the extraction buffer vial solution. It contains (0.5%) sodium azide as a preservative. Fatal if swallowed, in contact with skin or if inhaled. May cause damage to organs (Brain) through prolonged or repeated exposure if swallowed. When disposed of through sink, flush with a large quantity of water. Sodium azide has the potential to react with metals commonly found in the plumbing infrastructure, such as copper and lead, to form insoluble metallic azides – a highly explosive and shock sensitive compound.
6) Devices and extraction buffer bottle from different lot must not be used.
7) Do not use the test device if the pouch is not intact.
8) Do not use the nasopharyngeal swab, if found opened.
9) Do not use the test device if the desiccant color has changed from orange to green.
10) Do not smoke, eat or drink while handling specimens and performing a test.
11) Do not re-use the test device, nasopharyngeal swab and reaction buffer vial with nozzle as these are for single use only.
12) Perform the test by using kit extraction buffer solution, any other solution or fluid will invalidate the test results.
13) False Positive results may be possible, when insufficient extraction buffer is used in the test.
14) Use only nasopharyngeal swabs provided in the kit as other types of nasopharyngeal swabs have not been validated.

SPECIMEN COLLECTION

Procedural Notes

- Specimens collected in extraction buffer may be frozen at -20°C or are stable for 1 hour at room temperature before testing.
- Use only provided nasopharyngeal swab with kit for specimen collection.
- Collect the specimen wearing safety gloves and face mask to avoid contamination.
- Do not touch the minitip (specimen collection area) of the swab.

1) Nasopharyngeal (NP) swab collection: Insert minitip swab through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.

Note: *Specimen should be tested as soon as possible after collection. Specimens may be stored at room temperature for up to 1 hours prior to testing.*

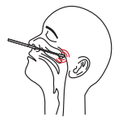
PICTORIAL PRESENTATION FOR SPECIMEN COLLECTION

NASOPHARYNGEAL SWAB COLLECTION

STEP 1

Tilt patient's head back 70 degrees. Insert a (Minitip) sterile swab into the nostril of the patient, swab over the surface of the posterior nasopharynx. (Swab should reach depth equal to distance from nostrils to outer opening of the ear).

STEP 2



Slowly rotate swab (right and left) in nostril to absorb secretions. Slowly remove swab after rotating it.

TEST PROCEDURE

1) Bring the Sure Status® COVID-19 Antigen Card Test kit components to room temperature(15°C to 30°C) prior to testing.
2) Open the device pouch, take out the test device from the aluminum pouch. Do not use the test device if the desiccant color has changed from orange to green. Label the test device with the patient identification number. Place the test device on a flat, clean, and dry surface.
3) Take extraction buffer bottle provided, twist open the cap and fill the reaction buffer vial upto the embossed marking or add 12 drops (Approx 300 µl) of extraction buffer into reaction buffer vial. Collect the Nasopharyngeal swab specimen with the help of Nasopharyngeal Swab provided inside the kit.
   Note: Please refer the Pictorial Presentation for Specimen collection.
4) Insert the Swab Specimen in the reaction buffer vial and swirl the swab 5-10 times.
5) Remove the swab while squeezing the sides of the tube to extract the liquid from the swab. Dispose of the used Nasopharyngeal Swab as biohazardous waste.
6) Close the nozzle cap tightly onto the reaction buffer vial by pressing. Invert the reaction buffer vial vertically and gently squeeze it to dispense 3 drops of specimens into a specimen well on the device and wait for 15-20 minutes for result.
   Note: Add the exactly 3 drops of extraction buffer as there is a possibility of False Positive results when insufficient extraction buffer is used in the test.
7) Do not interpret after 20 minutes.
   Note: If test window(Background) is not clear at 15 minutes then read the result at 20 minutes.
8) After recording the results, dispose of the test device and remaining reaction buffer vial solution as biohazardous waste.

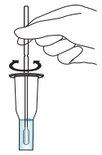
INTERNAL QUALITY CONTROL

The visualization of the Red colored control line in Sure Status® COVID-19 Antigen Card Test indicates that the active ingredient of the strips are functional and the migration is successful. The control line is a procedural control, serves to demonstrate functional reagents and correct migration of fluid. If the procedural control line does not develop in 20 minutes, the test result is considered invalid and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact the Technical Support at +91-260-2780112/113 (Available Hours: Mon. to Fri.: (08:00 to 17:00 IST) or info@premiermedcorp.com.

TEST PROCEDURE (PICTORIAL PRESENTATION)

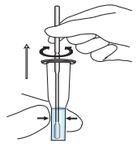
STEP 3

Take extraction buffer bottle provided, twist open the cap and fill the reaction buffer vial upto the embossed marking or add 12 drops (Approx 300 µl) of extraction buffer into reaction buffer vial.



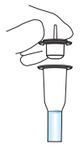
STEP 4

Insert the swab into an reaction buffer vial filled with extraction buffer. swirl the swab 5-10 times.



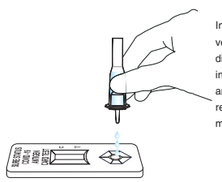
STEP 5

Remove the swab while squeezing the sides of the tube to extract the liquid from the swab.
Note: Dispose of the used Nasopharyngeal Swab as biohazardous waste.



STEP 6

Close the nozzle cap tightly onto the reaction buffer vial by pressing.

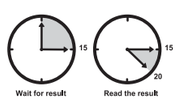


STEP 7

Invert the reaction buffer vial vertically and gently squeeze it to dispense 3 drops of specimens into a sample well of the device and wait for 15-20 minutes for result. Do not interpret after 20 minutes.

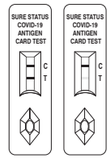
Results

Do not Interpret after 20 Minutes



HOW TO INTERPRET TEST RESULTS

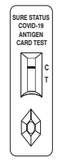
POSITIVE RESULTS



In addition to the presence of the C line, if the T line develops, the test indicates the presence of SARS-CoV-2 Antigen.
The result is positive or reactive.

Note: Interprete faint line as reactive line.
Alternative diagnosis method should be performed in order to obtain the confirmation of SARS-CoV-2 infections.

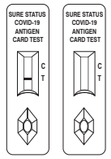
NEGATIVE RESULTS



If only a single line appears, at control line "C" as in the figure, the test indicates the absence of SARS-CoV-2 Antigen.

The result is Negative or non-reactive.

INVALID RESULTS



No presence of control line 'C' in the results window (irrespective of presence of test lines) indicates an invalid result.
The directions may not be followed correctly or the test may have detoriorated.
The Invalid test results should be retested with new test device.

PERFORMANCE CHARACTERISTICS

CLINICAL PERFORMANCE

Clinical performance characteristics of Sure Status® COVID-19 Antigen Card Test were evaluated in the USA and India. The study was carried out at 4 different sites. Testing was performed by qualified operators having laboratory experience. An FDA Emergency Use Authorized Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay for the detection of SARS-CoV-2 was used as the comparator method for this study.
In the study, the nasopharyngeal specimen were tested directly using the Sure Status® COVID-19 Antigen Card Test according to product instructions. While comparator method (rRT-PCR) was carried out using nasopharyngeal specimen eluted in viral transport media (VTM).

Sure Status® COVID-19 Antigen Card Test Performance against the Comparator Method

Sure Status® COVID-19 Antigen Card Test	Comparator Method		
	Positive	Negative	Total
Positive	129	00	129
Negative	08	837	845
Total	137	837	974

2 x 2 Contingency Table Statistical Analysis

Summary Statistics	Percent	95% Confidence interval
Sensitivity	94.16%	88.44% – 98.5%
Specificity	100.0%	99.43% – 100.0%

SURE STATUS® COVID-19 ANTIGEN CARD TEST PERFORMANCE AGAINST THE COMPARATOR METHOD (rRT-PCR)–BY THRESHOLD CYCLE (Ct) COUNTS

The performance of Sure Status® COVID-19 Antigen Card Test with positive nasopharyngeal swab specimens was validated using an FDA Emergency Use Authorized Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay. We correlated the results obtained for the 137 positive nasopharyngeal swab specimens with the comparator method (rRT-PCR) against Sure Status® COVID-19 Antigen Card Test. As presented in the table below, out of the 137 positive nasopharyngeal swab specimens, 106 samples had a Ct value 30. 31 out of 137 had a Ct value > 30. Our test was able to detect all 106 specimens having Ct value 30. Out of the 31 specimens having Ct value > 30, we correctly detected 23 specimens.

Sure Status® COVID-19 Antigen Card Test	Comparator Method positive by cycle threshold	
	Positive (Ct ≤ 30)	Positive (Ct >30)
Positive	106	23
Negative	00	08
Total	106	31
95% confidence interval	95.64% - 100%	55.07% - 100%

ANALYTICAL PERFORMANCE

LIMIT OF DETECTION (ANALYTICAL SENSITIVITY)

Limit of Detection (LOD) studies determine the lowest detectable concentration of SARS-CoV-2 at which 100% of all (true positive) replicates test positive. The LOD for the Sure Status® COVID-19 antigen rapid test was established using limiting dilutions of gamma-irradiated SARS-CoV-2 (BEI Resources; NR-52287, Lot# 70033322).
The NR-52287 is a preparation of SARS-Related Coronavirus 2 (SARS-CoV2), isolate USA WA1/2020, that has been inactivated by gamma-irradiation at 5 x 10^6 RADs. The material was supplied frozen at a concentration of 2.8 x 10^5 $TCID_{50}$/ml.
Presumed negative nasopharyngeal samples were diluted in PBS (supplemented with 0.05% Tween, pH=7.4) collected and combined to create a clinical nasal swab matrix. SARS-Related Coronavirus 2, Isolate USA WA1/2020, gamma irradiated was diluted in this natural nasal swab matrix pool to obtain the different viral dilutions required for the LOD study. Contrived nasal swab samples were prepared by absorbing 20 µl of each of the viral dilutions onto the swab. The swabs were tested according the testing procedure provided in the package insert.
Based on this study we found the tentative limit of detection (LOD) to be 22.4 $TCID_{50}$ / swab. The Final LOD was determined as the lowest virus concentration that was detected 95% of the time The concentration of 22.4 $TCID_{50}$ / swab was tested (20) times. Twenty (20) of twenty (20) tests were found to be positive for the concentration 22.4 $TCID_{50}$ / swab. Based on this testing the final limit of detection (LOD)was confirmed as 22.4 $TCID_{50}$ / swab.

Concentration $TCID_{50}$/Swab	Number Positive/Total	Detection%
22.4	20/20	100%

HIGH DOSE HOOK EFFECT

High Dose Hook Effect studies determine the level at which false-negative results can be seen when very high levels of a target are present in a tested sample. To determine if the Sure Status® COVID-19 antigen Test suffers from any high dose hook effect, increasing concentrations of gamma-irradiated SAR-SCoV-2 virus (BEI Resources NR-52287) were tested up to a concentration of 2.8 X 10^5 $TCID_{50}$/ml. In this study, the starting material was spiked into a volume of a pooled human nasopharyngeal matrices obtained from healthy donors and confirmed negative for SARS-CoV-2. At each dilution, 20 µL samples were added to swabs and the swabs processed for testing on the Sure Status® COVID-19 antigen Test as per the Product Insert using the procedure appropriate for patient nasopharyngeal swab specimens.
No impact on test performance or high dose hook effect was observed up to 2.8 X 10^5 $TCID_{50}$ / mL of gamma-irradiated SARS-CoV-2 with the Sure Status® COVID-19 antigen Test.

b) Instructions for Use of SS03-NS-P25

ENDOGENOUS INTERFERING SUBSTANCE STUDIES

A study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract in symptomatic subjects (including over the counter medications) do not cross-react or interfere with the detection of SARS-CoV-2 in the Sure Status® COVID-19 Antigen Card Test (Nasal Swab). Each substance was tested in triplicate in the absence or presence of SARS-CoV-2 at 75.6 $TCID_{50}$/Swab (3 X LOD).

No cross-reactivity or interference was seen with the following endogenous interfering substances when tested at the concentration presented in the table below:

Sr. No	Interfering Substance	Active Ingredient	Concentration
1	Endogenous	Mucin	2% w/v
2		Blood (human)	5% v/v
3	Nasal Gel	Sodium Chloride (NeilMed)	5% v/v
4	Nasal Drops	Phenylephrine	15% v/v
5	Nasal spray	Cromolyn	15% v/v
6		Oxymetazoline	15% v/v
7		Fluconazole	5% v/v
8	Homeopathic Nasal Spray	Alkalol	10% v/v
9		Fluticasone Propionate	0.5% v/v
10		Zincum gluconium (Zicam)	5% v/v
11	Sore Throat Phenol Spray	Phenol	15% v/v
12	Throat Lozenge	Benzocaine	0.15% v/v
13		Menthol	0.15 % v/v
14	Antiviral Drug	Zanamivir	300 ng/ml
15		Oseltamivir Phosphate (Tamiflu)	0.5% v/v
16	Antibiotic, systemic	Tobramycin	0.0004 % w/v
17	Antibiotic, Nasal ointment	Mupirocin	0.25 w/v
18	Allergy medication	Diphenhydramine (Benadryl)	0.1 % w/v
19	Anti- inflammatory medication	Dexamethasone	0.1 % w/v
20	Other substances	Biotin	100 µg/ml
21		HAMA	372 ng/ml
22		HAMA	297.2 ng/ml
23		Rheumatoid factor	4200 IU/ml

CROSS-REACTIVITY AND MICROBIAL INTERFERENCE STUDY (ANALYTICAL SPECIFICITY)

Cross-reactivity of the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) was evaluated by testing a panel of related pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen and could potentially cross-react with the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) including various microorganisms, viruses, and negative matrix.

Cross-Reactivity and potential interference of Sure Status® COVID-19 Antigen Card Test (Nasal Swab) was evaluated by testing sixteen (16) viruses, Thirteen (13) micro-organisms and two (2) negative matrixes. Each organism and virus were tested in triplicate in the absence or presence of gamma irradiated SARS-CoV-2 at 3 X the concentration of LOD (75.6 $TCID_{50}$/Swab). The final concentration of the organisms and viruses used in the study are documented in the table below (10^6 CFU/mL or higher for bacteria and 10^5 PFU/mL or higher for viruses was studied).

No cross-reactivity or interference was seen with the following microorganisms when tested at the concentration presented in the table below:

Sr. No	Potential Cross-Reactant		Concentration
1	Virus	MERS-CoV EMC/2012 (gamma-irradiated)	8.9 X 10^5 $TCID_{50}$/ml
2		Human coronavirus 229E	1 x 10^5 $TCID_{50}$/ml
3		Human coronavirus OC43	1 x 10^5 $TCID_{50}$/ml
4		Human coronavirus NL63	1.6 x 10^5 $TCID_{50}$/ml
5		Adenovirus, Type-07 (Species B)	1.0 x 10^5 PFU/mL
6		Human Metapneumovirus (hMPV)	1.0 x 10^5 $TCID_{50}$/ml
7		Parainfluenza virus Type 1	1.0 x 10^5 $TCID_{50}$/ml
8		Parainfluenza virus Type 2	1.0 x 10^5 $TCID_{50}$/ml
9		Parainfluenza virus Type 3	1.0 x 10^5 $TCID_{50}$/ml
10		Parainfluenza virus Type 4a	1.0 x 10^5 $TCID_{50}$/ml
11		Influenza A H3N2 (Wisconsin/67/05)	1.0 x 10^5 PFU/mL
12		Influenza A H1N1 (A/Brisbane/59/2007)	1.0 x 10^5 PFU/mL
13		Influenza B (Malaysia/2506/04)	1.0 x 10^5 PFU/mL
14		Enterovirus	1.0 x 10^5 PFU/mL
15		Respiratory syncytial virus Type A (RSV-A)	1.0 x 10^5 U/mL
16		Rhinovirus Type 1A	1.0 x 10^5 PFU/mL
17	Bacteria	*Haemophilus influenzae*	1.0 x 10^6 cells/mL
18		*Streptococcus pneumoniae*	1.0 x 10^6 CFU/mL
19		*Streptococcus pyogenes*	1.0 x 10^6 CFU/mL
20		*Bordetella pertussis*	1.0 x 10^6 cells/mL
21		*Mycoplasma pneumoniae*	1.0 x 10^6 CFU/mL
22		*Chlamydia pneumoniae*	1.0 x 10^6 IFU/mL
23		*Legionella pneumophila*	1.0 x 10^6 CFU/mL
24		*Mycobacterium tuberculosis*	1.0 x 10^6 CFU/mL
25		*Pneumocystis jirovecii*	1.0 x 10^6 CFU/mL
26		*Pseudomonas aeruginosa*	1.0 x 10^6 CFU/mL
27		*Staphylococcus epidermidis*	1.0 x 10^6 CFU/mL
28		*Streptococcus salivarius*	1.0 x 10^6 CFU/mL
29	Yeast	*Candida albicans*	1.0 x 10^6 cells/mL
30	Negative matrix	Universal Viral Transport Media	NA
31		Pooled human nasal wash collected for microbial flora	NA

LIMITATIONS

1) Negative results, should be treated as presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed.
2) Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
3) If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
4) Collected specimens may be frozen at -20°C and used up to 10 days after freezing and it is stable for 1 hour in extraction buffer at room temperature(15-30°C).
5) Results from antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
6) This test will indicate the presence of SARS-CoV-2 nucleocapsid protein antigen in the specimen from both viable and non-viable SARS-CoV-2 virus.
7) Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.
8) The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, storage, and preparation. Failure to perform proper procedures in any one of these steps can lead to incorrect results.
9) Results from the device should be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
10) This device has been evaluated for use with human specimen material only.
11) False-negative results may occur if the concentration of the target antigen in the clinical specimen is below the detection limits of the device.
12) This device is a qualitative test and does not provide information on the viral concentration present in the specimen.
13) This test cannot rule out diseases caused by other bacterial or viral pathogens.

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use		Contains sufficient for < n > tests
	Non Sterile	REF	Product Code
IVD	In vitro diagnostic medical device	LOT	Lot Number
4°C - 30°C	Store at 4-30 °C		Date of manufacture (YYYY-MM)
	Caution		Expiration Date (YYYY-MM)
	Keep dry		
	Do not reuse		

PRODUCT DISCLAIMER & WARNINGS

Every warnings and precaution should be taken into consideration before using the test. Failure to consider “Precaution, Warning, and Limitations” may not ensure the diagnostic ability and accuracy of this product. The test result may accordingly be affected by environmental factors and/or user error outside of the control of the Manufacturer and Distributor.

A definitive clinical diagnosis should not be based on the results of a single test, but it should be made by the physician after all clinical and laboratory findings have been evaluated.

“In no event shall our company or its distributor is liable for any direct, indirect, punitive, unforeseen, incidental, special consequential damages, to property or life, whatsoever arising out of or connected with an incorrect diagnosis, whether positive or negative, in the use or misuse of this product”.

In the event of performance changes or product malfunction, please contact manufacturer.

INSTRUCTIONS FOR USE

Follow the WHO/US CDC Universal Precautions for the safety against Novel Corona Virus (SARS-CoV-2).

Manufactured by

Premier Medical Corporation Private Limited

A1-302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA.
Customer support E-mail : info@premiermedcorp.com
Tel.: +91 2602780112/113 • Website : www.premiermedcorp.com

• ISO 13485 & EN ISO 13485 Certified Company

Part No.: SS03-NS-INS-001, Draft

ENGLISH

Note : Instructions for use will be printed in local language of the country using the test, if required.

SURE STATUS®

COVID-19 Antigen Card Test

Rapid Immunochromatographic Card Test for the detection of SARS-CoV-2 Antigen in Human Nasal Swab.

REF SS03-NS-P25

IVD 4°C - 30°C

INTENDED USE

Sure Status® COVID-19 Antigen Card Test (Nasal Swab) is a lateral flow immunochromatographic assay for qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in Nasal swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider.

Sure Status® COVID-19 Antigen Card Test (Nasal Swab) is for *in vitro* diagnostic use and intended as an aid to detection of nucleocapsid protein antigen in patient with clinical symptoms of SARS-CoV-2 infection. It provides only an initial screening test results and more specific alternative diagnosis method should be performed in order to obtain the confirmation of SARS-CoV-2 infections. The test is not automated and does not require any additional instrument. Test is designed to be performed by Laboratory professionals/trained users only.

INTRODUCTION

The novel coronavirus 2019-nCoV has recently emerged as a human pathogen in the city of Wuhan in China’s Hubei province, causing fever, severe respiratory illness, and pneumonia—a disease recently named COVID-19. According to the World Health Organization (WHO), as of 14 February 2021, there have been 108,153,741 confirmed cases of COVID-19, including 2,381,295 deaths, reported to WHO. The emerging pathogen was rapidly characterized as a new member of the betacoronavirus genus, closely related to several bat coronaviruses and severe acute respiratory syndrome coronavirus (SARS-CoV). Compared with SARS-CoV, 2019-nCoV appears to be more readily transmitted from human to human, spreading to multiple continents and leading to the WHO’s declaration of a Public Health Emergency of International Concern (PHEIC) on 30 January 2020. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more severe cases infection can cause pneumonia, severe acute respiratory syndrome (SARS), kidney failure and even death.

Coronavirus can be extracted through respiratory secretions or transmitted through oral fluid, sneezing, physical contact, and other air droplets. There are 2 main proteins involved and necessary for the infection of humans by a coronavirus. Which includes Spike protein (which is a trimeric protein containing 3 different sub-units, among three subunits, subunit S2 is highly conserved and subunit S1 is essential to make an entry into host cell) and nucleocapsid protein which is essential for viral assembly.

Thus these, 2 proteins play an important role in the infection and viral life cycle in human beings and are the potential target molecules for the development of a rapid test for the detection of coronavirus infection in humans. Sure Status® COVID-19 Antigen Card Test (Nasal Swab) uses nucleocapsid protein as a measurand and particularly, used for the identification of subclinical or asymptomatic cases. It also plays a role in reducing or preventing the transmission of infection as these individuals may transmit the virus. As a Rapid Diagnostic Test with a 15-20 min testing time, Sure Status® COVID-19 Antigen Card Test (Nasal Swab) could be effectively used at a large scale during population screening of COVID-19 infection.

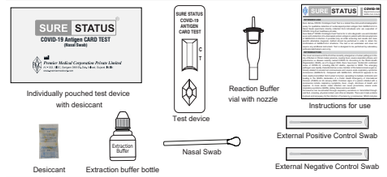
ASSAY PRINCIPLE

The detection kit uses the principle of immunochromatography: separation of components in a mixture through a medium using capillary force and the specific and rapid binding of an antibody to its antigen. Each cassette is a dry medium that has been coated separately with anti-coronavirus (anti Nucleocapsid) antibody molecule, this can be a monoclonal or a poly clonal antibody directed against nucleocapsid protein of 2019-nCoV and goat anti-chicken IgY antibody (control line). 2 free colloidal gold labeled antibodies, i.e anti-nucleocapsid antibody as well as chicken IgY, will be sprayed on the conjugate pad. Once specimen as a Nasal swab is collected and diluted in extraction buffer, this diluted specimen will be applied on sample pad. The specimen in buffer will pass through the conjugate pad and bind with anti-nucleocapsid antibody conjugated with colloidal gold on the conjugate pad and will form a complex of antigen-antibody colloidal gold and will migrate towards test and control lines. Thus, the formed complex of antigen-antibody-colloidal gold will migrate through capillary action and binds with the coated antibody molecules at the test line, thus providing a reactive result.

If there is no formation of antigen-antibody colloidal gold complex, it will not bind with the test line and there will not be any development of test lines. Chicken IgY conjugated with colloidal gold will bind with control line antibody irrespective of reactive/non-reactive specimens.

MATERIALS PROVIDED	PACK SIZE : 25 TESTS
Test device pouch containing: test device and desiccant	25 Nos.
Nasal swab	25 Nos.
Reaction buffer vial with nozzle	25 Nos.
Extraction Buffer Bottle	5 Nos.
Instructions for use	1 No.
External Positive Control swab	1 No.
External Negative Control swab	1 No.

MATERIALS PROVIDED



MATERIALS REQUIRED BUT NOT PROVIDED

- New pair of disposable gloves & facemask
- Pen
- Timer
- PPE Kits
- Biohazardous waste container
- Extra Nasal swab, if needed

STORAGE AND STABILITY

1) Sure Status® COVID-19 Antigen Card Test (Nasal Swab) kit should be stored at 4-30°C.
2) Do not freeze the kit or components.
3) The kit is sensitive to humidity and heat. Do not store the kit at temperatures above 30°C and in humid conditions.
4) Extraction Buffer Bottle (opened and unopened) & the unopened test device are stable until the expiry date printed on the label, when stored at 4-30°C.
5) Perform the test within 15 minutes after removing the test device from the aluminium pouch. If a desiccant color has changed from orange to green, do not use the test device.
6) The test device is stable until the printed expiry date on the pouch/external secondary packaging.

PRECAUTIONS

1) Wear protective clothing, masks, gloves and take other appropriate safety precautions to avoid or reduce the risk of infection while handling of specimen.
2) Dispose of used gloves as biohazard waste. Wash hands thoroughly afterward.
3) Avoid splashing or aerosol formation.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Decontaminate and dispose of all used specimens, test devices, Nasal swab, Extraction buffer bottle and Reaction buffer vial with nozzle in a biohazardous waste container.
6) Sure Status® COVID-19 Antigen Card Test (Nasal Swab) kit requires the handling of human specimens. It is recommended that all human-sourced materials and all consumables contaminated with potentially infectious materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.

WARNINGS

1) For *in vitro* diagnostic use only.
2) The test has been authorized only for the presence of specific antigen to SARS-CoV-2 and not for any other viruses or pathogens.
3) Read the instructions carefully before performing the test, any deviation will invalidate the test results.
4) Apply standard biosafety precautions for handling and disposal of potentially infective materials including human biological specimens irrespective of disease state.
5) Do not drink the reaction buffer vial/extraction buffer solution. It contains a preservative which may be toxic if ingested. When disposed of through sink, flush with a large quantity of water.
6) Devices and Extraction buffer bottle from different lot must not be used.
7) Do not use the test device if the pouch is not intact.
8) Do not use the Nasal swab, if found opened.
9) Do not use the test device if the desiccant color has changed from orange to green.
10) Do not smoke, eat or drink while handling specimens and performing a test.
11) Do not re-use the test device, Nasal swab and reaction buffer vial with nozzle as these are for single use only.
12) Perform the test by using kit extraction buffer solution, any other solution or fluid will invalidate the test results.

SPECIMEN COLLECTION

Procedural Notes

- Process the test sample immediately after collection.
- Use only provided or recommended Nasal swab for specimen collection.
- Collect the specimen wearing safety gloves to avoid contamination.
- Do not touch the tip (specimen collection area) of the swab.

- Nasal Swab collection: Tilt patient's head back 70 degrees. Use the swab provided inside the kit. While gently rotating the swab, insert it less than one inch (about 2 cm) into nostril parallel to the palate until resistance is met at turbinates. Rotate the swab several times against nasal wall. Remove swab, insert it into the other nostril and repeat the process.

Note: *Specimen should be tested as soon as possible after collection. Specimens may be stored at room temperature for up to 1 hour prior to testing.*

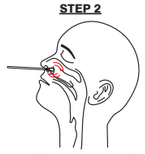
PICTORIAL PRESENTATION FOR SPECIMEN COLLECTION

NASAL SWAB COLLECTION

STEP 1

Tilt patient's head back 70 degrees. While gently rotating the swab, insert it less than one inch (about 2 cm) into nostril parallel to the palate until resistance is met at turbinates.

STEP 2



Rotate the swab several times against nasal wall.
Remove swab, insert it into the other nostril and repeat the process.

TEST PROCEDURE

1) Bring the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) kit components to room temperature prior to testing.
2) Remove the test device and the other kit components from kit and place them on a flat, clean and dry surface.
3) Take extraction buffer bottle provided, twist open the cap and fill the reaction buffer vial upto the embossed marking or add 12 drops (Approx 300 µl) of extraction buffer into reaction buffer vial. Collect the Nasal Swab specimen with the help of Nasal Swab provided inside the kit.
   Note: Please refer the Pictorial Presentation for Specimen collection.
4) Insert the Swab Specimen in the reaction buffer vial and swirl the swab 5-10 times. Dispose of the used Nasal Swab as biohazardous waste.
5) Remove the swab while squeezing the sides of the reaction buffer vial with nozzle to extract the liquid from the swab. Close the nozzle cap tightly onto the reaction buffer vial by pressing.
6) Invert the reaction buffer vial and gently squeeze it to draw 3 drops of specimens into a sample well on the device and wait for 15-20 minutes for result. Do not interpret the result after 20 minutes.
   Note: If test window (Background) is not clear at 15 minutes then read the result at 20 minutes.
7) After recording the results, dispose of the test device and remaining reaction buffer vial solution as biohazardous waste.

INTERNAL QUALITY CONTROL

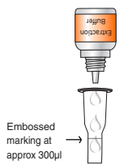
The visualization of the Red colored control line in Sure Status® COVID-19 Antigen Card Test (Nasal Swab) indicates that the active ingredient of the strips are functional and the migration is successful. The control line is a procedural control, serves to demonstrate functional reagents and correct migration of fluid.If the procedural control line does not develop in 15 minutes, the test result is considered invalid and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact the Technical Support at +91-260-2780112/113 (Available Hours: Mon. to Fri.: (08:00 to 17:00 IST) or info@premiermedcorp.com.

EXTERNAL QUALITY CONTROL

External Control: External control is used to demonstrate that the test device and test protocol perform properly. It is recommended that external positive and negative control swabs are run once with every new kit. External positive and negative control swabs are provided in the kit.

Note: The external controls should be tested using the nasal swab test procedure provided in this IFU.

TEST PROCEDURE (PICTORIAL PRESENTATION)

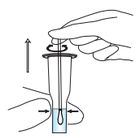


STEP 3

Take extraction buffer bottle provided, twist open the cap and fill the reaction buffer vial upto the embossed marking or add 12 drops (Approx 300 µl) of extraction buffer into reaction buffer vial.

STEP 4

Insert the swab into a reaction buffer vial filled with extraction buffer. swirl the swab 5-10 times.

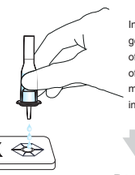


STEP 5

Remove the swab while squeezing the sides of the reaction buffer vial to extract the liquid from the swab.

STEP 6

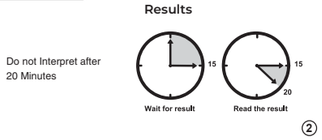
Close the nozzle cap tightly onto the reaction buffer vial by pressing.



STEP 7

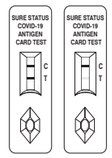
Invert the reaction buffer vial and gently squeeze it to draw 3 drops of specimens into a sample well of the device and wait for 15-20 minutes for result. Do not interpret after 20 minutes.

Results



HOW TO INTERPRET TEST RESULTS

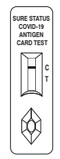
POSITIVE RESULTS



In addition to the presence of the C line, if the T line develops, the test indicates the presence of SARS-CoV-2 Antigen.
The result is positive or reactive.

Note: Interprete faint line as reactive line.
Alternative diagnosis method should be performed in order to obtain the confirmation of SARS-CoV-2 infections.

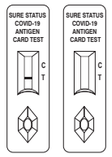
NEGATIVE RESULTS



If only a single line appears, at control line "C" as in the figure, the test indicates the absence of SARS-CoV-2 Antigen.

The result is Negative or non-reactive.

INVALID RESULTS



No presence of control line 'C' in the results window (irrespective of presence of test lines) indicates an invalid result.
The directions may not be followed correctly or the test may have detoriorated.
The Invalid test results should be retested with new test device.

PERFORMANCE CHARACTERISTICS

CLINICAL PERFORMANCE

Clinical performance characteristics of Sure Status® COVID-19 Antigen Card Test (Nasal Swab) were evaluated in India. The study was carried out at 3 different sites. Testing was performed by qualified operators having laboratory experience. An FDA Emergency Use Authorized Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay for the detection of SARS-CoV-2 was used as the comparator method for this study.
In the study, nasal swabs were tested directly using the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) according to product instructions. The other two swabs (i.e. nasopharyngeal and oral) were eluted in viral transport media (VTM) and sent to the clinical laboratory for rRT-PCR for the comparator method study.

Sure Status® COVID-19 Antigen Card Test (Nasal Swab) Performance against the Comparator Method

Sure Status® COVID-19 Antigen Card Test (Nasal Swab)	Comparator Method		
	Positive	Negative	Total
Positive	159	01	160
Negative	17	478	495
Total	176	479	655

2 x 2 Contingency Table Statistical Analysis

Summary Statistics	Percent	95% Confidence interval
Sensitivity	90.34%	84.99% – 94.27%
Specificity	99.79%	98.84% – 99.99%

SURE STATUS® COVID-19 ANTIGEN CARD TEST (NASAL SWAB) PERFORMANCE AGAINST THE COMPARATOR METHOD – BY CYCLE THRESHOLD COUNTS

The performance of Sure Status® COVID-19 Antigen Card Test (Nasal Swab) with positive nasal swab specimens was validated using an FDA Emergency Use Authorized Real-time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR) assay. We correlated the results obtained for the 176 positive nasal swab specimens with the comparator method (rRT-PCR) against Sure Status® COVID-19 Antigen Card Test (Nasal Swab). As presented in the table below, out of the 176 positive nasal swab specimens, 126 samples had a Ct value 30. 50 out of 176 had a Ct value > 30. Our test was able to detect all 126 specimens having Ct value 30. Out of the 50 specimens having Ct value > 30, we correctly detected 33 specimens. This shows that our test is able to accurately detect positive nasal swab specimens having a low viral count.

Sure Status® COVID-19 Antigen Card Test (Nasal Swab)	Comparator Method positive by cycle threshold	
	Positive (Ct ≤ 30)	Positive (Ct > 30)
Positive	126	33
Negative	00	17
Total	126	50
95% confidence interval	97.11% - 100%	51.23% - 78.79%

ANALYTICAL PERFORMANCE

LIMIT OF DETECTION (ANALYTICAL SENSITIVITY)

Limit of Detection (LOD) studies determine the lowest detectable concentration of SARS-CoV-2 at which 100% of all (true positive) replicates test positive. The LOD for the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) was established using limiting dilutions of gamma-irradiated SARS-CoV-2 (BEI Resources; NR-52287, Lot #70033322).
The NR-52287 is a preparation of SARS-Related Coronavirus 2 (SARS-CoV-2), isolate USA WA1/2020, that has been inactivated by gamma-irradiation at 5 x 10^6 RADs. The material was supplied frozen at a concentration of 2.8 x 10^5 $TCID_{50}$/ml.
Presumed negative nasal samples were diluted in PBS (supplemented with 0.05% Tween, pH=7.4) collected and combined to create a clinical nasal swab matrix. SARS-Related Coronavirus 2, Isolate USA WA1/2020, gamma irradiated was diluted in this natural nasal swab matrix pool to obtain the different viral dilutions required for the LOD study. Contrived nasal swab samples were prepared by absorbing 20 µl of each of the viral dilutions onto the swab. The swabs were tested according the testing procedure provided in the package insert.
Based on this study we found the tentative limit of detection (LOD) to be 25.2 $TCID_{50}$/swab. The Final LOD was determined as the lowest virus concentration that was detected 95% of the time the concentration of 25.2 $TCID_{50}$/swab was tested (20) times. Twenty (20) out of twenty (20) tests were found to be positive for the concentration 25.2 $TCID_{50}$/swab. Based on this testing the final limit of detection (LOD)was confirmed as 25.2 $TCID_{50}$/swab.

Concentration $TCID_{50}$/Swab	Number Positive/Total	Detection%
25.2	20/20	100%

HIGH DOSE HOOK EFFECT

High Dose Hook Effect studies determine the level at which false-negative results can be seen when very high levels of a target are present in a tested sample. To determine if the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) suffers from any high dose hook effect, increasing concentrations of gamma-irradiated SARS-CoV-2 virus (BEI Resources NR-52287) were tested up to a concentration of 2.8 X 10^5 $TCID_{50}$/ml. In this study, the starting material was spiked into a volume of a pooled human nasal matrices obtained from healthy donors and confirmed negative for SARS-CoV-2. At each dilution, 20 µL samples were added to swabs and the swabs processed for testing on the Sure Status® COVID-19 Antigen Card Test (Nasal Swab) as per the Product Insert using the procedure appropriate for patient nasal swab specimens.
No impact on test performance or high dose hook effect was observed up to 2.8 X 10^5 $TCID_{50}$ / mL of gamma-irradiated SARS-CoV-2 with the Sure Status® COVID-19 Antigen Card Test (Nasal Swab).