ENDOGENOUS INTERFERING SUBSTANCES STUDIES
A study was performed to determine the 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. Each substance was tested in triplicate in the absence or presence of SARS-CoV-2 at 67.2-70.2 TCID50/mL (SA LD50).

LIMITATIONS
1) No cross-reactivity or interference should be treated as presumptive and confirmatory 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 the accuracy of the result.
3) If the differentiation of specific SARS viruses and strains is needed, additional testing may be required.
4) A negative result in an individual with clinical signs of infection may require further testing to exclude SARS-CoV-2 infection.
5) Collected specimens may be frozen at 4°C and up to 10 days after freezing and is stable for 1 hour in extraction buffer at room temperature (4-30 °C).
6) Results from the antigen test should be used solely for the basis of diagnosis or exclusion of SARS-CoV-2 infection or to determine infection status.
7) This test will not be able to detect the presence of viral disease in asymptomatic patients, exonerating the patient.
8) The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, processing, and preparation. Failure to observe proper procedures in any of these steps can lead to incorrect results.
9) Results from the device should be correlated with the clinical history, epidemiologic, and other laboratory results for the determination of the diagnosis. (exam exonerating the patient)
10) The 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 test is not a diagnostic test of the presence of detectable amount of SARS-CoV-2 in the sample and may not correlate with viral culture results performed on the same sample.
13) The detection of SARS-CoV-2 nucleocapsid antigen is dependent upon proper specimen collection, handling, processing, and preparation. Failure to observe proper procedures in any of these steps can lead to incorrect results.
14) This test cannot rule out the presence of other bacterial or viral pathogens.
15) Test may result in cross-reactivity with other strains like human rhinovirus HKU1 and SARS-CoV or SARS-CoV-1.
16) False-negative results may occur due to high concentration of antibody (Hook Effect).

REFERENCES
3) Cross-reactivity and potential interferents of Sure Status® COVID-19 Antigen Card Test was evaluated by testing a panel of related pathogens, high prevalence disease agents, and normal or clinical flora that are reasonably likely to be confused in the clinical specimen and could potentially cross-react with the Sure Status® COVID-19 Antigen test including various microorganisms, viruses, and negative matrix.
4) The Sure Status® COVID-19 Antigen Card Test kit requires the handling of human samples and materials and all human samples and materials are contaminated with potentially infectious materials which are considered potentially infectious and in accordance with the OSHA Standard on Bloodborne Pathogens, Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are contaminated with, or are contaminated with infectious agents.
5) In vitro diagnostic use only.
6) Do not freeze the kit or components.
7) The kit is sensitive to humidity and heat. Do not store the kit at temperatures above 40°C or below 4°C.
8) 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.
9) Process the test immediately (within 15 minutes) after removing the test device from the vial. A change in the test device color from descendent color has changed from orange to green do not use the test device.
10) Wear protective clothing, masks, gloves and take other appropriate safety precautions to avoid or reduce the risk of infection while handling specimens.
11) Dispose of used gloves as biohazard waste. Wash hands thoroughly afterward.
12) Avoid any contact with eyes or mouth.
13) Clean up spills thoroughly using an appropriate disinfectant.
14) Do not use the test device with all used specimens, test devices, nasopharyngeal swab, Extraction buffer bottle and Reaction buffer vial with nozzle, in a laboratory with existing devices.
15) Sure Status® COVID-19 Antigen Card Test kit requires the handling of human specimens and all human samples and materials are contaminated with potentially infectious materials which are considered potentially infectious and in accordance with the OSHA Standard on Bloodborne Pathogens, Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are contaminated with, or are contaminated with infectious agents.

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 swab specimens directly collected from individuals suspected of COVID-19 by their healthcare provider.

PRODUCT DISCLAIMER & WARNINGS
The test result should not be considered a guarantee of the presence of SARS-CoV-2. In the event of performance changes or product modification, please contact manufacturer.

INSTRUCTIONS FOR USE
Follow the VAGOUS GDC Universal Precaution for the safety against VAGOUS GDC (VAGOUS GDC-VAGOUS GDC).

Manufactured by
Premier Medical Corporation Private Limited A1-302, GIDC, Sanand-380015, Dist. Vadodara, Gujarat, INDIA. Tel: +91 2692701213 / 14 Website: www.premiermedcorporation.com

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Part No.: S029-INS-001, Rev.: DRAFT

MATERIALS PROVIDED

Sure Status® COVID-19 Antigen Card Test kit should be stored at 4-30°C.

If do not freeze the kit or components.

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 40°C or below 4°C.
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) Process the test immediately (within 15 minutes) after removing the test device from the vial. A change in the test device color from descendent 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 any contact with eyes or mouth.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Do not use the test device with all used specimens, test devices, nasopharyngeal swab, Extraction buffer bottle and Reaction buffer vial with nozzle, in a laboratory with existing devices.
6) Sure Status® COVID-19 Antigen Card Test kit requires the handling of human specimens and all human samples and materials are contaminated with potentially infectious materials which are considered potentially infectious and in accordance with the OSHA Standard on Bloodborne Pathogens, Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are contaminated with, or are contaminated with infectious agents.

Warnings
1) In vitro diagnostic use only.
2) Do not perform the test 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 result.
4) Apply standard biosafety precautions for handling and disposal of potentially infectious 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 exposure may cause respiratory irritation. If swallowed, may cause damage to organs (Brain). May be toxic if swallowed. Avoid contact with eyes. May cause irritation of the eyes. Wash eyes with大量 of water. Sodium azide may form explosive metal azides – a highly explosive and shock sensitive compound. C7H8N2 + 4H2SO4  => 4SO2 + 2N2 + 7H2O + 6H2, an explosive reaction. Equipment and extraction buffer bottle from different lot must not be used.
6) Do not use the test device if the pouch is not intact.
7) Do not use the nasopharyngeal swab, if found broken.
8) Do not use the test device if the descent color has changed from orange to green.
9) Do not smoke, or eat while handling specimens and performing a test.
10) Do not reuse the test device, nasopharyngeal swab and reaction buffer vial with nozzle as these are for single use only.
11) Do not use test device if the pouch is not intact.
12) Do not use the nasopharyngeal swab, if found broken.
13) Always wear the appropriate safety glasses and face mask to avoid contamination.
14) Do not touch the strip (specimen collection area) of the swab.
TEST PROCEDURE (PICTORIAL PRESENTATION)

PICTORIAL PRESENTATION FOR SPECIMEN COLLECTION

Nasopharyngeal Swab Collection

1) Bring the Sure Status® COVID-19 Antigen Card Test kit components to room temperature (19°C to 32°C) prior to testing.
2) Open the device pouch, take out the test device from the aluminum pouch. Do not open the kit up to 20 minutes prior to testing.

TEST PROCEDURE

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.

STEP 3

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

STEP 4

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.

STEP 5

Close the nozzle cap tightly onto the reaction buffer vial by pressing. Invert the reaction buffer vial to extract the liquid from the swab. Dispose of the used Nasopharyngeal Swab as biohazardous waste.

STEP 6

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

STEP 7

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

STEP 8

Invert the reaction buffer vial vertically and gently squeeze it to dispense 3 drops of specimen into a specimen well on the device and wait for 15-20 minutes for result.

RESULTS

No presence of control line ‘C’ in the results window (prespective of presence of test line) indicates an invalid result.

The directions may not be followed correctly or the test may have deteriorated.

The invalid test results should be retested with new test device.

INSTRUCTIONS

The visualisation 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 precautionary function 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 result, 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.

How to interpret test 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: Interpretate line as reactive line.

Alternative diagnosis method should be performed in order to obtain the confirmation of SARS-CoV-2 infections.

Performance characteristics

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 Authorization 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 specimens 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

<table>
<thead>
<tr>
<th>Sure Status® COVID-19 Antigen Card Test</th>
<th>Comparator Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>129</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
</tr>
</tbody>
</table>

Sure Status® COVID-19 Antigen Card Test vs. Comparator Method

<table>
<thead>
<tr>
<th>Sure Status® COVID-19 Antigen Card Test</th>
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<tr>
<td>Positive</td>
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</tr>
<tr>
<td>Total</td>
<td>129</td>
</tr>
</tbody>
</table>

2 x 2 Contingency Table Statistical Analysis

Results

<table>
<thead>
<tr>
<th>Summary Statistics</th>
<th>Percent</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94.16%</td>
<td>88.44% - 98.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100.0%</td>
<td>99.63% - 100.0%</td>
</tr>
</tbody>
</table>

Sensitivity 94.16%, Specificity 100.0%.

Analytical performance

Limit of Detection (Analytical Sensitivity)

The performance of Sure Status® COVID-19 Antigen Card Test with positive nasopharyngeal swab specimens was validated using an FDA Emergency Use Authorization 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 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 Performance against the Comparator Method by Threshold Cycle (Ct) Counts

Analytical Performance

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 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 105 Rad. Surrogate Nasopharyngeal Swab was prepared by adding a 15 µl aliquot of the resuspended virus in 0.05% Tween, pH=7.4 to a swab. Presumed negative nasopharyngeal samples were diluted in PBS (supplemented with 0.05% Tween, pH=7.4) and combined to create a clinical nasal swab matrix. SARS-Related Coronavirus 2, isolate USA-WA1/2020, gamma-irradiated 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 adding 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 TCID50 / swab. The Final LOD was determined as the lowest virus concentration that was detected ≥95% of the time. The concentration of 22.4 TCID50 / swab was tested (20) times. Twenty (20) or (20) tests were found to be positive for the concentration 22.4 TCID50 / swab. Based on this testing the final limit of detection (LOD) was confirmed as 22.4 TCID50 / swab.