INTRODUCTION

**Coviscreen™** is an in vitro, rapid, self performing, qualitative, Double Antigen sandwich immunoassay for the simultaneous detection of Total antibodies (IgM+IgG+IgA) to SARS-CoV-2 virus in serum, plasma, and whole blood. It is to be used for screening or to aid in the diagnosis of COVID-19 disease and exposure to the virus. Double Antigen Sandwich assays are also known to detect the antigen specific IgA isotype antibodies and contribute to detect the seroconversion in patient samples earlier as compared to the other tests as detection of Total antibodies (IgM+IgG+IgA) is possible.

**Coviscreen™** uses Double Antigen sandwich immunochromatography method for the detection of total antibodies (IgM+IgG+IgA) to SARS-CoV-2 virus in human serum/plasma/whole blood, collected from individuals showing clinical signs and symptoms of the COVID-19 infection or with the history of travelling or residing in a geographical region with active COVID-19 infections as declared in the CDC SARS-CoV-2 virus clinical and epidemiological criteria. Being a screening test for COVID-19 infection, presumptive positive specimens with anti-SARS-CoV-2 IgM and/or IgG and/or IgA antibodies need to be further confirmed with additional tests recommended by CDC/ICMR for the diagnosis of COVID-19 infection.

SUMMARY

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new coronavirus, that caused the outbreak of infections in Wuhan in 2019. Coronaviruses (CoV) are a large family of enveloped viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

Coronaviruses are zoonotic and are transmitted between animals and people and are commonly found in many different species of animals, including camels, cattle, cats, and bats. SARS-CoV-2 is a new strain that was discovered in 2019 and had not been previously identified in humans. WHO has officially named the disease as Corona Virus Disease 2019 (COVID-19). Cases have been detected in most countries worldwide and community spread is being detected in a growing number of countries. WHO has declared the COVID-19 outbreak as a pandemic. SARS-CoV-2, the causative viral agent of the disease COVID-19, is a coronavirus which bears the transmembrane glycoprotein spikes (S protein) typical of the viruses in its clade. These spikes are a prominent target of human immune responses and have been found to be highly immunogenic. The receptor binding domain of the S protein is particularly targeted by the neutralizing antibodies. The spikes on the SARS-CoV-2 allows the virus to enter the host cells through the human receptor angiotensin converting enzyme 2 (ACE2), present in the alveolar epithelial cells. The time between initial viral exposure and symptom onset is known as incubation period. Antibodies can appear on an average as soon as three days post-exposure or as late as thirteen days post exposure. The most characteristic symptom of COVID-19 patients is respiratory distress. Most people infected with SARS-CoV-2 virus do not have symptoms, but when present they are usually mild and last less than seven days. Common symptoms of COVID-19 infection are fever, headache,nausea and vomiting. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Older people and people with severe chronic conditions are at higher risk of developing serious COVID-19 illness.

During the first 14 days after onset of symptoms, COVID-19 disease can be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) in samples of symptomatic patients. Virus-specific IgM, IgA and IgG antibodies are typically present after the first four days of illness and may be detectable, for up to 12 weeks. As SARS-CoV-2 is a newly emerging virus, there is an unusual pattern observed in the antibody response. Studies have shown in this particular infection, Total antibodies (IgM+IgA+IgG) are detected first, followed by IgM and IgG. Total antibodies is a term used when IgM, IgA and IgG antibodies are detected together without differentiation. Combined with patient demography and clinical findings, detection of Total antibodies (IgM+IgA+IgG) antibodies for SARS-CoV-2 virus provides an essential tool for diagnosing and following up an acute or recent infection. There is currently no available vaccine or proven anti-viral drug treatment for SARS-CoV-2 virus.

PRINCIPLE

Total Antibodies (IgM+IgG+IgA) specific to Spike protein of SARS-CoV-2 virus are detected in this test. **Coviscreen™** utilizes the principle of agglutination of antibodies with respective antigen in immuno-chromatography format along with use of nano indicator colloidal particles as agglutination revealing agent. Recombinant antigens specific to SARS-CoV-2 are captured as capture in the Test region “T” and biotinylated bovine serum albumin as assay control in the control region “C”. As the test specimen flows through the membrane assembly within the test device, the colored—SARS-CoV-2 specific recombinant antigen—indicator colloidal particles complexes with SARS-CoV-2 antibodies, if present in the specimen. This complex moves further on the membrane to the Test region where it is immobilized by the SARS-CoV-2 recombinant antigens coated as capture on the nitrocellulose membrane leading to formation of a colored band in the Test region “T” which confirms a positive test result. Absence of the colored band in the Test region “T” indicates a negative test result.

The unreacted conjugate and the unbound complex, if any along with the streptavidin-colloidal gold conjugate move further on the membrane and are subsequently immobilized by the biotinylated bovine serum albumin coated at the control region “C”, forming a colored band. This control band serves to validate the test results.

REAGENT AND MATERIAL SUPPLIED

**Coviscreen™** test kit comprises of:

A. Individual pouches, each containing:
   1. **DEVICE** : Membrane assembly pre-dispensed with SARS-CoV-2 virus specific recombinant antigen-indicator colloidal particles, streptavidin gold conjugate, SARS-CoV-2 virus specific recombinant antigen capture at test region “T” and Biotinylated BSA at control region “C”.
   2. Desiccant pouch.
B. **Sample running buffer in a dropper bottle. Physiological buffer containing stabilizers and preservatives.**
C. Package Insert.
**TEST PROCEDURE**

1. Bring the Coviscreen™ kit components to room temperature before testing.
2. Open the foil pouch by tearing along the "notch".
3. Retrieve the device and desiccant pouch. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard that test device and use another device.
4. Once opened, the device must be used immediately.
5. Tighten the cap of the sample running buffer bottle provided with the kit in clockwise direction to pierce the bottle nozzle.
7. Place the device on a flat horizontal surface.
8. **Specimen addition**
   - 8.1 For venous whole blood/serum/plasma samples: Using the sample dropper provided carefully dispense 20 µl into the specimen port 'A'. Kindly note that the sample should be aspirated upto the appropriate graduated mark on the dropper. Alternatively, using a precision micropipette, carefully add 20 µl specimen into specimen port 'A'.
   - 8.2 Finger prick whole blood: Gently clean the pricking area using Alcohol swab and allow it to air dry. Prick the area using sterile lancet and wipe off first ooze of blood with swab. Gently squeeze the finger so that blood oozes out and collect the blood using sample dropper up to 20 µl mark and immediately dispense 20 µl on to specimen port 'A'.
   - 9. Next add four drops of sample running buffer into the buffer port (B) and immediately start the stopwatch.
   - 10. Read the final result at the end of 20 minutes. Do not interpret results after 30 minutes

**For institutional packs: Follow this procedure for specimen addition**

Specimen addition:
   - 8.1 Venous whole blood/serum/plasma: Using micropipette provided carefully dispense 20 µl of specimen at the specimen port 'A'.
   - 8.2 Finger prick whole blood: Gently clean the pricking area using Alcohol swab and allow it to air dry. Prick the area using sterile lancet and wipe off first ooze of blood with swab. Gently squeeze the finger so that blood oozes out and collect the blood using the micropipette provided and immediately dispense 20 µl on to specimen port 'A'.

**NOTE**

1. Finger prick/whole blood sample should be tested immediately.
2. As the micropipette provided is 10 µl. For whole blood sample including finger prick, the sample volume for testing is 20 µl. Therefore the whole blood sample will have to be aspirated 2 times (10 µl) using this micropipette and the same to be dispensed into the specimen port 'A'.

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**STORAGE AND STABILITY**

The test kit (including sealed pouches) may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE the kit or its components. After first opening of the sample running buffer bottle, it can be stored between 4°C to 30°C for remaining duration of its shelf life.

**NOTE**

(1). For in vitro diagnostic use and for professional use only. NOT FOR MEDICINAL USE. (2). Do not use the kit beyond expiry date and do not re-use the test device. (3). Read the instructions carefully before performing the test. (4). Any modification to the above procedure and/or use of other reagents will invalidate the test results. (5). Do not mix the reagent or devices from different lots. (6). Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation/swallowing may cause harm. (7). Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material. (8). Sample running buffer contains Sodium Azide (~ 0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

**SPECIMEN COLLECTION AND PREPARATION**

1. No special preparation of the patient is necessary prior to specimen collection by approved techniques.
2. Though fresh serum/plasma is preferable, specimen may be stored at 2°C to 8°C for up to 24 hours in sterile condition, in case of delay in testing.
3. For whole blood sample, collect blood in a tube containing EDTA as anticoagulant. It should be tested immediately after sample collection.
4. As an alternative to sample dropper provided; a 20 µl well calibrated micropipette may be used, Stop watch, Disposable gloves, Blood collection device and accessories.

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INTERPRETATION OF RESULT

Negatives result:
The presence of only one pink-purple coloured band in the control area marked ‘C’, indicates the absence of specific antibodies against SARS-CoV-2 virus or that the amount of antibodies is below the detection limit of the test.

Positive Results:
In addition to the band in the control area marked ‘C’, appearance of a pink-purple coloured band in the test region ‘T’, indicates the presence of SARS-CoV-2 virus specific Total (IgM+IgG+IgA) antibodies.

Invalid Result:
The test result is invalid if no bands appear on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE EVALUATION

Internal Evaluation
1. Sensitivity: In an in-house study, the performance of Coviscreen™ was evaluated using a panel of 27 nos. RT-PCR positive specimens, Coviscreen™ showed positive in all 27 specimens. The results of the evaluation are as follows:

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>Coviscreen</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

2. Specificity: A total of 188 known COVID-19 negative samples were tested with Coviscreen™. Specificity of 99.07% was observed.

LIMITATIONS OF THE TEST
(1). Coviscreen™ detects the presence or absence of IgM/IgG/IgA antibodies to SARS-CoV-2 virus in the human serum/plasma/whole blood specimen. It should not be used as sole criteria for the treatment and management of COVID-19 infection. Detection of IgM isotype makes the test more sensitive. (2). Colour intensity of the test band is directly proportional to the antibodies present in the sample, hence it may vary from very light-coloured band to high intense coloured band. (3). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated. (4). This test can give positive results after successful treatment. Therefore, this test is not recommended for monitoring response to treatment. (5). A negative result with Coviscreen™ does not always preclude the sero-status of the infection of COVID-19. Patient should be re-tested after 3-4 days in case of clinically non-correlated result for further confirmation and should be confirmed by real-time reverse transcriptase-polymerase (RT-PCR) method. (6). Serological cross-reactivity across the other Coronavirus group may occur in certain patients with prior exposure to HKU1 or NL63 or OC43 or 229E or SARS-CoV or MERS-CoV etc. (7). There is always a possibility that false results may occur due to presence of interfering substances in the specimen or factors beyond the control of the manufacturer, such as technical or procedural errors associated with the testing. (8). Do not interpret the test results beyond 30 minutes. (9). This test does not differentiate between the current infection and past infection. (10). This test is meant for and validated for testing human’s serum/plasma/whole blood samples only. (11). This test is not meant for testing of pooled samples. (12). Hemoglobin levels ≤ 5 mmol/L, Triglycerides ≤ 5 mmol/L, Bilirubin levels ≤ 500 mg/L do not interfere with the results. (13). The immunocompetence of the patient, viral dose on exposure play a role in generation of the antibody response. (14). In an interference study, human serum positive for antibodies to dengue, HIV, HCV, Toxoplasma, Zika and syphilis, along with Influenza A & B, Hepatitis B and Malaria positive specimens did not interfere with test results.

WARRANTY
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY
INSTRUCTIONS FOR ASSEMBLING THE DISPOSABLE SAMPLE DROPPER

1. Components

The sample dropper consists of 2 parts (refer Fig 1)
(a) Bulb
(b) Graduated Microtip

2. Assembly

1. Open the pouch and remove the graduated microtip and the bulb
2. As shown in the Fig 2. Hold the bulb in one hand and the graduated microtip in the other hand, slide the narrow end of the bulb into the wide mouth of microtip till the bulb gets fitted into the microtip properly. (Fig.3).

USER INSTRUCTIONS (for Finger prick sample)

1. Clean finger to be pricked with an alcohol swab. Allow to dry.
2. Prick the finger with the pointed end of the sterile lancet.
3. Aspirate the blood using a freshly assembled dropper (by half pressing the bulb of the dropper and releasing it) up to the required mark.
4. Check the level of blood absorbed on the microtip indicating 20 µl.
5. Dispense the 20 µl blood on the test device as mentioned in the specimen addition.

For venous whole blood/serum/plasma aspirate sample from the collection tube and follow step (4) and (5) of this pictorial.
Same procedure is to be followed for the one-piece sample dropper or minipipette as provided.

Manufactured by: Zephyr Biomedicals
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