VALIDATION OF SARS COVID-19

(Nasopharynx samples: 30 positive samples and 30 negative samples were collected from patients.)

RT-PCR tests were run using Thermofisher Multiplex Gold Std method. Using the same samples that were collected from patients we collected samples for Rapid Sars Covid-19 Antigen rapid test. For in vitro qualitative direction of SARS-coV-2 nucleocapsid antigen in nasal (NS) swab specimens directly from individuals who are suspected of COVID-19 by their healthcare provider within the first 5 days after onset symptoms. This test is only provided for use by clinical laboratories or to healthcare workers for point-of-care testing, not for at-home testing. Severe acute respiratory syndrome coronavirus 2 (SARS-coV-2) is an enveloped non-segmented positive sense RNA virus. It is the cause of coronavirus disease (COVID-19), which is contagious in humans. SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N) The antigen is generally detectable in upper respiratory samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but the clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out a bacterial infection or coinfection with other viruses. The agent detected may not be the definite cause of disease. Negative results should be treated as presumptive, which do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19 and confirmed with a molecular assay, if necessary for patient management.

JOYSBIO Biotechnology's SARS CoV-2 Antigen Rapid Test Kit uses an immunocapture method, it is designed to detect the presence of absence of SARS-CoV-2 nucleocapsid proteins in respiratory samples from patients with signs and symptoms of infection who are suspected of COVID-19. Key components: the anti-nucleocapsid protein antibody and chicken IgY labeled by colloidal gold the nitrocellulose membrane coated with anti-nucleocapsid protein antibody, and

goat anti -chicken IgY antibody. When specimens are processed and added to the test device, SARS-CoV-2 antigens present in the specimen bind to antibodies conjugated to colloidal gold in the test strip. The antigen- conjugate complexes migrate across the test strip to the reaction area and are captured by a line of antibodies bound on the membrane. A color band will show up when antigen- conjugate is deposited at the test "T" position and the control "C" position on the device.

Materials Provided:

Component	20 Tests/Box	40 Test/Box	Main Components
Test Device	20 Tests/Box (1 Test/pouch x 20 Pouches)	40 Tests/Box (1 Test/Pouch x 40 Pouches)	The anti-nucleocapsid protein antibody and chicken IgY labeled by colloidal gold, the nitrocellulose membrane coated with anti nucleocapsid protein antibody and goat anti-chicken IgY antibody
Desiccant	20 Packs	40 Packs	Silica Gel
Buffer	20 single-use bottles, each with 350 mL extraction buffer	40 single-use bottles, each with 350 mL extraction buffer	Detergent Solution
Extraction Tube	20 single use reaction tubes, each with 1x nozzle cap	40 single use reaction tubes, each with 1x nozzle cap	/
Specimen Sampling Swabs	20 sterile, single use specimen sampling swabs	40 sterile, single use specimen sampling swabs	/

SARS-CoV-2 (+) Control Swab	1 each - individually wrapped for single use	Non-infectious recombinant viral protein antigen with less than 0.1% sodium azide.

SARS- CoV-2 (-) Control Swab	1 each - individually wrapped for single use	Buffer with less than 0.1% sodium azide

Specimen Collection and Preparation:

Acceptable specimens for testing with this kit include nasal swab specimens obtained by the dual nares collection method. Correct specimen collection and preparation methods must be followed. Specimens obtained early during symptom onset will contain the highest viral titers; specimens obtained after five days of symptoms are more likely to produce negative results when compared to an RT-PCR assay. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, training in specimen collection is highly recommended due to the importance of specimen quality for generating accurate test results.

Specimen Transport and Storage:

Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection. Correct specimen collection and preparation methods must be followed.

Nasal Swab Specimen Collection:

a. Insert swab into one nostril of the patient. The swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nostril. Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected.

b. Using the same swab, repeat this process for the other nostril to ensure that an adequate sample is collected from both nasal cavities.

c. With draw the swab from the nasal cavity. The sample is now ready for processing using the kit.

Test Procedure:

- **Step 1:**

Twist off the top of the buffer bottle, slowly dispense all of the buffer into the extraction tube,

- **Step 2:**

After collection of nasal (NS) swab specimen,, insert the swab into the tube and plunge the swab up and down in the fluid for a minimum of **20 seconds**, then hold the swab against the bottom of the tube and **roll 5 turns,** taking care not to splash contents out of the tube.

- **Step 3:**

Remove the swab while squeezing the sides of the tube to extract the liquid from the swab.

- **Step 4:**

Press the nozzle cap firmly onto the extraction tube containing the processed sample (threading or twisting is not required). Mix thoroughly swirling or flicking the bottom of the tube. Place the extraction tube (s) in a rack in the designated area of the workplace.

- **Step 5:**

Tear off the foil pouch, take out the test strip/cassette and place the test kit on a clean and level surface. Label the test device and one extraction tube for each specimen or control to be tested.

- **Step 6:**

Gently squeeze the rigid body of the tube, dispensing **three (3) drops** of the processed specimen into the sample well.

- **Step 7:** Read the test results between **15 and 20 minutes.** Do not read the results after **20 minutes.**

Validation Results

	RTPCR	ANTIGEN	NEGATIVE PTS
1. **5009**	**20.8**	+	**Negative**
2. **5021**	**20.9**	+	**Negative**
3. **5031**	**22.1**	Wk +	**Negative**
4. **38**	**22.0**	Wk +	**Negative**

5. **44**	**18.81**	+	**Negative**
6. **48**	**15.2**	+	**Negative**
7. **72**	**23.3**	**Wk+**	**Negative**
8. **78**	**23.9**	**+**	**Negative**
9. **92**	**20.4**	**+**	**Negative**
10. **100**	**12.4**	**+**	**Negative**
11. **206**	**18.7**	**+**	**Negative**
12. **210**	**23.6**	**Wk +**	**Negative**
13. **225**	**16.3**	**+**	**Negative**
14. **256**	**18.8**	**+**	**Negative**
15. **264**	**20.8**	**+**	**Negative**
16. **290**	**13.8**	**+**	**Negative**
17. **301**	**17.3**	**Wk +**	**Negative**
18. **323**	**18.7**	**+**	**Negative**
19. **333**	**18.2**	**+**	**Negative**
20. **335**	**19.8**	**+**	**Negative**
21. **388**	**22.8**	**+**	**Negative**
22. **404**	**15.1**	**V +**	**Negative**
23. **551**	**11.3**	**+**	**Negative**

24. **564**	**25.4**	**+**	**Negative**
25. **572**	**17.4**	**+**	**Negative**
26. **578**	**16.9**	**+**	**Negative**
27. **646**	**29.2**	**Wk +**	**Negative**

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28. **648**	**21.1**	+	**Negative**
29. **651**	**13.2**	+	**Negative**
30. **671**	**17.7**	+	**Negative**

100% MATCHING WITH RT-PCR USING GOLD STANDARD THERMO FISHER MULTIPLEX METHOD.

Virus MKU 1