三和生物科技有限公司
Sanwa BioTech Ltd

- ***For in vitro diagnostic use***
- ***This test has been validated but FDA's independent review is pending***

ALiA

SARS-CoV-2 Antigen FIA Test

Intended Use

ALiA SARS-CoV-2 Antigen FIA Test is a fluorescent immunoassay intended for the qualitative detection of nucleocapsid antigen from SARS-CoV-2 in nasopharyngeal swab from individuals who are suspected of COVID-19 infection by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests and point-of-care sites that are covered by the laboratory's CLIA certificate for high complexity.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. Antigen is generally detectable in nasopharyngeal swab during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results 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.

The ALiA SARS-CoV-2 Antigen FIA Test is intended for use with ALiA FIA Analyser by clinical laboratory technicians.

Symbols

REF	Catalogue number		Manufacturer
LOT	Batch code / lot number	Rx Only	For prescription use only

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Prescription use only.
- To be used in conjunction with the ALiA FIA Analyser.
- For optimal result, use the 1 mL COPAN UTM provided as part of the test.
- Do not use expired test.
- Do no reuse test, this product is of single-use only.
- Check product for integrity before use. Do not use damaged product.
- Performed test in an area with adequate ventilation.
- Consider all human samples as capable of transmitting infectious diseases and handle them with care. Wear suitable protective clothing, gloves, and eye/face protection when handling human samples and reagents. It is recommended to follow biosafety level 2 or other appropriate safety practices when handling samples.
- Treat all reagents as harmful and handle with care.
- Dispose biochip, containers and any unused reagents in accordance with local regulatory requirements.

Limitations

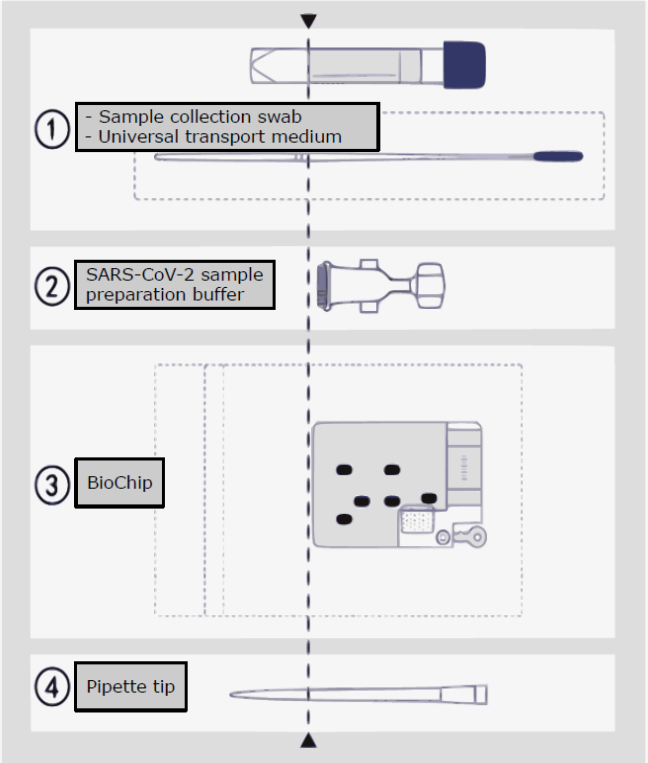
- ALiA SARS-CoV-2 Antigen FIA Test was validated with samples stored in 1-3 mL of COPAN UTM. Performance of test cannot be guaranteed if excess volume of UTM is used or VTM from other brands is used.
- Cross-reactivity with SARS-CoV has not been determined.
- ALiA SARS-CoV-2 Antigen FIA Test detects both viable (live) and non-viable SARS-CoV-2 viruses. Test performance depends on the amount of virus (antigen) in the sample and may or may not compare with viral culture results performed on the same sample.

- The detection of viral antigen is highly dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to follow proper procedures in any one of these steps may lead to incorrect results.
- This test is a qualitative test and does not provide information on the viral load present in the sample.
- Results from the ALiA SARS-CoV-2 Antigen FIA Test should be correlated with the patient's clinical history, epidemiological data and other data available to evaluate the patient.
- This test has been evaluated for use with human sample material only.
- False negative results may occur if the viral level in the clinical sample is below the detection limits of the device.
- If the virus mutates in the target region, SARS-CoV-2 virus may not be detected or may be detected less predictably.
- The performance of this test has not been evaluated for patients without signs and symptoms of SARS-CoV-2 infection.
- The performance of this test has not been evaluated for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The performance of this test has not been evaluated for the screening of blood or blood derivatives for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The effect of interfering substances has only been evaluated for those listed in the labelling. Interference by substances other than those described below can lead to erroneous results.
- Cross-reactivity with respiratory tract organisms other that those listed in the labelling may lead to erroneous results.
- The prevalence of infection will affect the test's predictive value. False negative test results are likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low SARS-CoV-2 activity when prevalence is moderate to low.

Reagents and Materials

Materials Provided

1. Instructions for use × 1
2. Quick start guide × 1
3. Test pack × 10, each containing:
   - ① Sample collection swab × 1
     Universal transport medium × 1
   - ② SARS-CoV-2 sample preparation buffer × 1
   - ③ BioChip × 1
   - ④ Pipette tip × 1



Materials Required but not Provided

- ALiA FIA Analyser (model DX320, catalogue number: 10101)
- ALiA SARS-CoV-2 Antigen FIA Test External Positive Control (catalogue number: 5014-01)
- Volumetric pipette or precision pipette capable of delivering 50 µL of liquid.

Storage Conditions

- Store in the dark at 4 °C
- Avoid direct sunlight
- Do not freeze
- Use the BioChip as soon as possible once removed from the sealed foil package
- Use all buffers immediately after opening the vial caps to avoid contamination
- Use sample collection swab immediately after removing from its primary packaging to avoid contamination
- The test kits are stable until the expiration date as shown on the label

Summary

SARS-CoV-2 is commonly known as the COVID-19 virus. The SARS-CoV-2 virus was first identified in Wuhan, China in December 2019. The origin of this virus is currently unknown but it is thought that it originated from bats. With the rapidly climbing infection rate, the WHO declared COVID-19 a pandemic disease on 11 March 2020. Millions of confirmed infections have been seen globally.

COVID-19 patients may show infection symptoms or can be asymptomatic. Symptomatic patients are likely to show symptoms similar to other respiratory viruses infection. Some of the commonly seen symptoms are fever, fatigue, dry cough, sputum production, and shortness of breath. Symptoms are expected to appear within 14 days of infection with the median incubation time estimated to be around 5 days.

Principle of the Test

The ALiA SARS-CoV-2 Antigen FIA Test is based on the traditional fluorescent immunoassay technique. This test has been fully automated and miniaturized to minimize running time and possibility of human errors.

A nasopharyngeal swab sample should be collected from patient exhibiting COVID-19 infection symptoms and subsequently stored in viral transport medium. The nasopharyngeal swab stored in viral transport medium can be used for further confirmation tests such as cell culture. The addition of elution buffer dilutes any mucus that might be present in the sample and the lysis buffer releases SARS-CoV-2 viruses from cells.

The highly-integrated BioChip contains pre-loaded reagents and an immobilized fluorescent immunoassay microarray. After the patient sample is loaded into the sample reservoir, the BioChip should be inserted into the ALiA FIA Analyser. The ALiA FIA Analyser controls the reagent flow, incubation time, and automatically analyzes the fluorescent signal detected from the microarray.

ALiA SARS-CoV-2 Antigen FIA Test uses a combination of SARS-CoV-2 viral antigen-specific monoclonal and polyclonal antibodies that can effectively detect SARS-CoV-2 nucleocapsid antigen in nasopharyngeal samples. However, negative result does not preclude the possibility of an infection as factors such can microbial contamination, poor sample quality and presence of uncommon interfering substance may lead to performance deterioration and so it is recommended that negative results be confirmed by an FDA molecular test or by viral culture.

Quality Control

Each ALiA SARS-CoV-2 Antigen FIA Test has internal positive and negative controls embedded in the immunoassay microarray. A test will be flagged as invalid if the ALiA FIA Analyser fails to detect these internal control signals.

External positive control and negative control are available as a separate product (ALiA SARS-CoV-2 Antigen FIA Test External Control Pack, catalogue number 5014-01). Each kit contains 1 vial of positive external control and 1 vial of negative control. Run the external controls in accordance to the test procedures outlined for samples in this user manual.

It is recommended to run external positive control and external negative control:

- for each new, untrained operator
- once for each new shipment of products to ensure the different lot received in the shipment
- when performance deterioration is suspected
- according to your quality management procedures and internal quality control measures

Test procedures

The procedures outlined here are specific for the ALiA SARS-CoV-2 Antigen FIA Test. For general instructions on operation and maintenance of the ALiA FIA Analyser, refer to the ALiA FIA Analyser user manual.

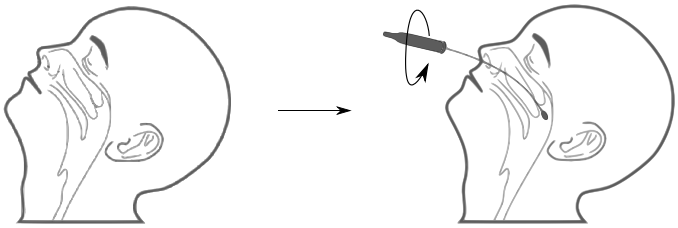
Step 1: Sample collection at collection site

Proper sample collection and preparation are crucial to accurate antigen detection. Inadequate sample volume, inappropriate sample collection, inappropriate transport conditions, or inappropriate storage conditions can adversely affect the test performance.

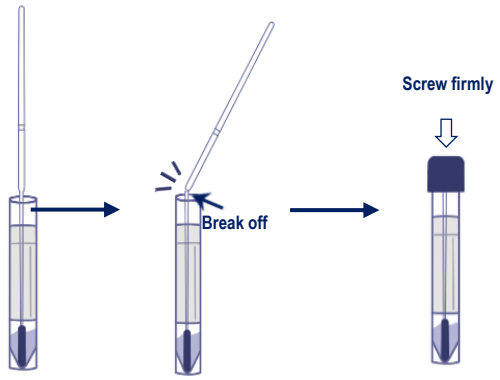
Samples should be tested as soon as possible after collection to yield the best test performance. Store the collected sample in the universal transport media provided and keep at 4 °C throughout transportation and storage. The sample is stable in the universal transport media at 4 °C for up to 48 hours.

Nasopharyngeal Swab Sample Collection

1. Tilt patient's head back 70 degrees.
2. Choose the nostril that presents the most secretion under visual inspection.
3. Insert the swab through the nostril and advance along the floor of the nose until it reaches the nasopharynx.
4. Leave the swab in place for several seconds to absorb secretions. Slowly remove the swab while rotating it.



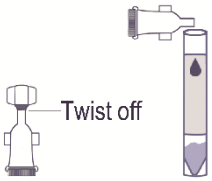
5. Put the swab into the Universal transport medium (UTM) and snap off the tip of the swab at the red mark (break point). Screw cap firmly with the swab inside of the vial. Dispose the remaining part of the swab. The sample should be stored at 4 °C.



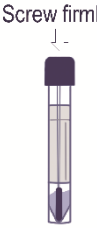
Step 2: Sample preparation at laboratory

1. Take out the BioChip from cold storage 20 minutes prior to use to allow the Sample preparation buffer and the BioChip to reach room temperature.

2. Twist off the cap of Sample Preparation Buffer. Dispense all buffer into the inoculated UTM and follow the sample collection process.

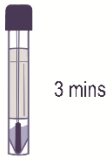


3. Screw on the cap. Make sure the cap is tightly screwed.



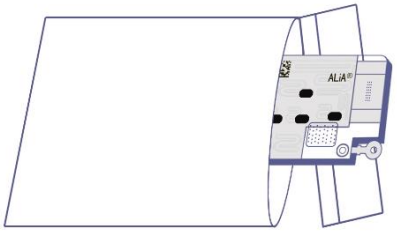
4. Vigorously shake the UTM for 10 seconds to break up mucus and homogenize the inoculated UTM mixture.

5. Let the sample rest at upright position for 3 minutes.

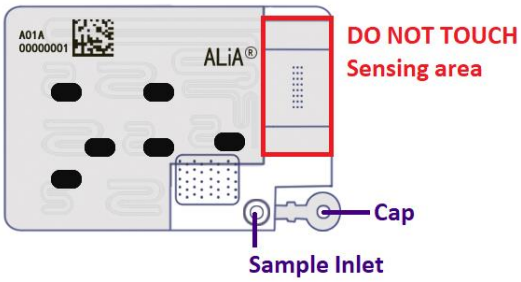


Step 3: Take out BioChip

1. Take the BioChip out of the foil bag just before testing.

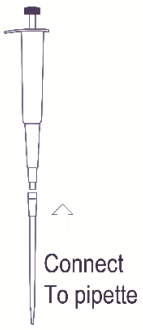


*** Caution: DO NOT touch the Sensing area**

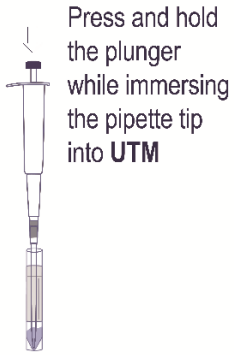


Step 4: Sample loading

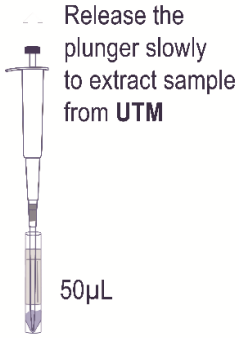
1. Attach the Pipette Tip provided to the tip cone of the pipette. Be careful not to contaminate the pipette tip.



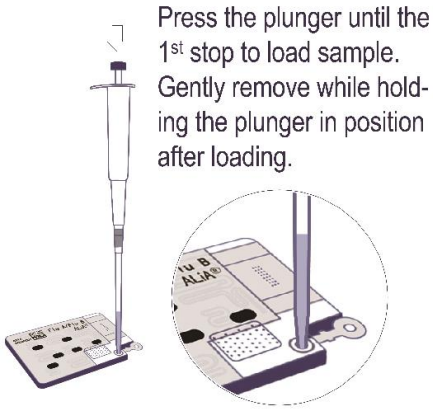
2. Press the pipette plunger until 1st stop is hit. Hold the plunger and immerse the pipette tip into the inoculated UTM. Make sure the pipette tip is at the bottom of the vial.



3. Slowly release the pipette plunger to extract 50 µL of sample. Avoid inclusion of any blood or mucus clots in the pipette tip.

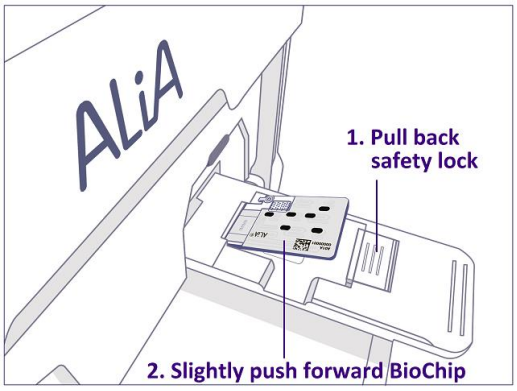


4. Sample loading
   a. Hold the pipette so it is perpendicular to the BioChip. Carefully insert the Pipette Tip into the sample inlet.
   b. Inject the sample by slowly pressing the pipette plunger until the 1st stop.
   c. CONTINUE TO HOLD THE PLUNGER DOWN after loading is completed and gently remove the Pipette Tip from the BioChip.
   d. Close the cap firmly.

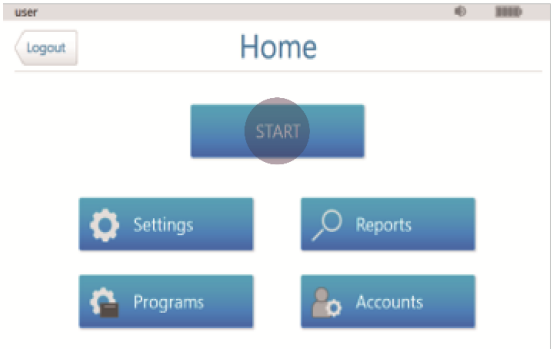


Step 5: Running the test on ALiA FIA Analyser (DX320)

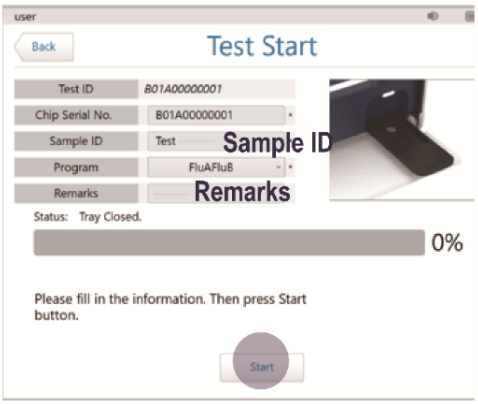
1. Insert BioChip into the ALiA FIA Analyser tray
   a. Pull out the tray
   b. Pull the safety lock backward and slide the BioChip into the BioChip Slot.
   c. Release the safety lock to secure the BioChip.
   d. Push the tray back into the ALiA FIA Analyser.



2. Refer to the ALiA FIA Analyser user manual for full operating instructions.
   a) On the home page, press START to enter test page.



   b) When "*Chip Detected*" is displayed on screen, serial number should be detected automatically. If serial number for the BioChip is not displayed, manually enter the serial number located next to the 2D barcode on the BioChip.
   c) The Program is chosen automatically. If the program fails to do so, choose the Program manually.
   d) Enter sample ID (2 – 50 characters in length). Enter remarks if necessary (up to 255 characters in length).



***Caution: DO NOT pull out the tray during test.**

Step 6: Interpret Results

ALiA FIA Analyser automatically analyses and interprets microarray signals once the test has been completed. Final results are displayed on screen. Remove the BioChip and dispose it according to your local regulations.

Positive result
- Presence of antigen in the sample.
- Does not rule-out other respiratory viral co-infections.

Negative result
- Presumptive negative for the presence of antigens.
- Does not exclude viral infection.
- Viral culture or PCR is suggested for confirmation.

Invalid result
- Any of the built-in positive or negative control signals are not detected.
- Likely to indicate a product problem as opposed to sample problem
- If the control test performs as expected, a new test on the sample should be performed with a new BioChip.

Analytical Performance Characteristics

Limit of detection

Limit of detection of the ALiA SARS-CoV-2 Antigen FIA Test was determine by limiting dilution studies using serial dilutions of the characterized SARS-CoV-2 culture fluid (Zeptometrix). The culture fluid is a preparation of SARS-related coronavirus 2 (SARS-CoV-2), isolate Hong Kong/VM20001061, that has been inactivated by heating at 65 °C for 30 minutes. The material was supplied frozen at a concentration of 3.16×10^6 $TCID_{50}$ / mL.

The limit of detection study was designed to reflect the assay when using nasopharyngeal swabs inoculated into UTM. In order to mimic the sample collection and preparation process, a swab was spiked with approximately 50 µL of the virus dilution in saline. The spiked swab was added to the UTM containing pooled negative NP matrix. The inoculated UTM was subsequently tested in accordance to the ALiA SARS-CoV-2 Antigen FIA Test user manual.

Based on this methodology, serial dilutions of the culture fluid were tested in triplicates. The lowest concentration at which all three replicates were positive was treated as the tentative limit of detection. The LoD was then confirmed by 17 extra testing with concentrations at the tentative limit of detection. The final LoD of the test was determined to be the lowest concentration resulting in positive detection in at least 19/20 tests. Based on this methodology, the limit of detection chosen was 510 $TCID_{50}$ / mL.

Inclusivity

Inclusivity was demonstrated using the strains USA/WA1/2020 and Italy/INMI1. The minimum detectable levels for USA/WA1/2020 was 4.87×10^4 $TCID_{50}$ / mL and for Italy/INMI1 was 3.08×10^3 $TCID_{50}$ / mL.

Cross reactivity & Microbial Interference

Cross reactivity and microbial interference of ALiA SARS-CoV-2 Antigen FIA Test was evaluated using a total of 32 non-SARS-CoV-2 respiratory pathogens and other microorganisms. Bacterial and yeast species are evaluated at the concentrations equivalent to 10^6 cfu/mL or higher while viral species are evaluated at the concentrations equivalent to 10^5 pfu/mL or higher.

All tests gave SARS-CoV-2 antigen negative and so no cross-reactivity was seen at the tested concentrations.

All tests gave SARS-CoV-2 antigen positive when samples were spiked with 3x LoD of SARS-CoV-2 and so no microbial interference was observed.

Organism	Concentration
Human coronavirus 229E	1×10^5 pfu/mL
Human coronavirus OC43	2×10^5 pfu/mL
Human coronavirus NL63	1×10^5 pfu/mL
MERS-coronavirus (Florida/USA-2_Saudi Arabia_2014)	1×10^5 pfu/mL
Influenza A H1N1pdm (California/07/09)	2×10^5 pfu/mL
Influenza A H3N2 (Singapore/INFIMH-16-0019/16)	2×10^5 pfu/mL
Influenza B (Colorado/06/17)	2×10^5 pfu/mL
Influenza B (Phuket/3073/13)	2×10^5 pfu/mL
Respiratory Syncytial Virus Type A (RSV-A) (3/2015 Isolate 3)	2×10^5 pfu/mL
Respiratory Syncytial Virus Type B (RSV-B) (Strain: CH93(18)-18)	2×10^5 pfu/mL
Adenovirus Type 01 (Species C)	2×10^5 pfu/mL
Adenovirus Type 03 (Species B)	2×10^5 pfu/mL
Adenovirus Type 04 (Species E)	2×10^5 pfu/mL
Adenovirus Type 07 (Species B)	2×10^5 pfu/mL
Parainfluenza Virus Type 1	2×10^5 pfu/mL
Parainfluenza Virus Type 2	2×10^5 pfu/mL
Parainfluenza Virus Type 3	2×10^5 pfu/mL
Parainfluenza Virus Type 4B	2×10^5 pfu/mL
Rhinovirus (Isolate: 10/2014 Isolate #1)	2×10^5 pfu/mL
Enterovirus Type 68 (2014 Isolate)	2×10^5 pfu/mL
Human Metapneumovirus 16 Type A1	2×10^5 pfu/mL
Mycoplasma pneumoniae M129	2×10^6 cfu/mL
Streptococcus pyogenes Z018	2×10^6 cfu/mL
Haemophilus influenzae type b	2×10^6 cfu/mL
Legionella pneumophila Philadelphia	2×10^6 cfu/mL
Streptococcus pneumoniae 19F Z022	2×10^6 cfu/mL
Bordetella pertussis A639	2×10^6 cfu/mL
Pneumocystis jiroveci-S. cerevisiae	2×10^6 cfu/mL
Candida albicans	2×10^6 cfu/mL
Staphylococcus aureus	2×10^6 cfu/mL
Staphylococcus epidermidis	2×10^6 cfu/mL
Chlamydia pneumoniae	2×10^6 cfu/mL

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of SARS coronavirus that was not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology, and the likelihood of cross reactivity was evaluated.

- For Human Coronavirus HKU1, homology exists between the SARS- CoV-2 nucleocapsid protein and Human Coronavirus HKU1. BLAST results showed 30 sequence IDs, all nucleocapsid protein, showing homology. Sequence ID AGW27840.1 had the highest alignment score and was found to be 38.7% homologous across 76% of the sequences, this is relatively low but cross-reactivity cannot be fully ruled out.

- For SARS-Coronavirus, high homology exists between the SARS-CoV-2 nucleocapsid protein and SARS-Coronavirus. BLAST results showed 68 sequence IDs, mostly nucleocapsid protein, showing homology. Sequence ID AAR87518.1, had the highest alignment score isolated from a human patient and was found to be 90.76% homologous across 100% of the sequence. This is high and cross-reactivity is likely to happen.

- For Pneumocystis jirovecii (PJP), the homology between SARS-CoV-2 nucleocapsid protein and Pneumocystis jirovecii total protein (3,745 proteins) is relatively low, homology-based cross-reactivity can be ruled out.

- For Mycobacterium tuberculosis, the homology between SARS-CoV-2 nucleocapsid protein and Mycobacterium tuberculosis total protein (3,991 proteins) is relatively low, homology-based cross-reactivity can be ruled out.

Interference

Potential interfering substances such as blood, over-the-counter drugs are evaluated using ALiA SARS-CoV-2 Antigen FIA Test. All tests give SARS-CoV-2 antigen negative results at the tested concentrations, and positive results when 3x LoD of SARS-CoV-2 was present in the sample, showing that there is no interference with the substances tested.

Substance	Concentration
Whole blood	4%
Mucin	0.5%
Chloraseptic (Menthol/Benzocaine)	1.5
Naso GEL (NeilMed)	5% v/v
Nasal Drops (Phenylephrine)	15% v/v
Logicin (Oxymetazoline)	15% v/v
Nasal Spray (Cromolyn)	15% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Sore Throat Phenol Spray	15% v/v
Tobramycin	4
Mupirocin	10
Fluticasone Propionate	5% v/v
Biotin	3500
Tamiflu (Oseltamivir Phosphate)	5

Clinical Performance Characteristics

The performance of the ALiA SARS-CoV-2 Antigen FIA Test was established in a retrospective, randomized, and blinded clinical performance evaluation study. Commercial samples bought from the US were used in the study. All samples were pre-characterized by an FDA EUA RT-PCR test kit. Only nasopharyngeal swab samples eluted in UTM were evaluated in this study.

75 nasopharyngeal swab samples were sourced commercially and 68 nasopharyngeal swab samples were tested. 7 nasopharyngeal swab samples were excluded from the study due to not meeting the sample inclusion criteria and no tests were performed on these excluded samples. Out of the 68 samples tested, 65 samples were included in data analysis and 3 of invalid results were excluded from data analysis. The positive percent agreement of ALiA SARS-CoV-2 Antigen FIA Test is 86.7% and the negative percent agreement is 100%.

ALiA SARS-CoV-2 Antigen Test compared to FDA EUA PCR				
		PCR		
		+	–	Total
ALiA	+	26	0	26
	–	4	35	39
Total		30	35	65
PPA		= 26/30 × 100% = 86.7% (95% CI 70.3 – 94.7%)		
NPA		= 35/35 × 100% = 100% (95% CI 90.1 – 100%)		

Technical Support

If you have questions regarding the use of this product or if you would like to report a problem, please contact your local distributor for technical support. Alternative, you may want to directly contact us via support@sanwabiotech.com or call us at +852 2698 6612, 10:00 – 18:00 (GMT+8) Monday to Friday.

REF 5013-10

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