Shenzhen Lvshiyuan Biotechnology Co.,Ltd

Green Spring®SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold)
Instructions for Use

REF GF102B1L Rev. 7.1	English

Rapid test for the qualitative detection of SARS-CoV-2 nucleocapsid antigen. For professional use.

INTENDED USE

The Green Spring® SARS-CoV-2 Antigen Rapid Test is intended for the rapid qualitative detection of SARS-CoV-2 nucleocapsid protein antigen in human saliva, nasal, nasopharyngeal or oropharyngeal swab samples. The results are used for the detection of SARS-CoV-2 antigen. The antigen is generally detectable in upper respiratory tract samples during the acute phase of infections. Positive results do not exclude bacterial infection or co-infection with other viruses. The pathogen detected may not be the sole cause of the disease.
Negative results should be treated as suspected cases and confirmed with a molecular assay. Negative results should be considered in the context of a patient's recent exposures, history and presence of clinical signs and symptoms consistent with COVID-19. The test should only be performed by trained medical personnel.

SUMMARY

The novel coronaviruses belong to a ß-genus. COVID-19 is an acute respiratory infectious disease. Humans are generally susceptible to it. Currently, patients infected with the novel coronavirus are the main source of infection; asymptomatically infected people may also be a source of infection. The main manifestations include fever, fatigue and a dry cough. A stuffy or runny nose, sore throat, muscle aches and diarrhoea occur in a few cases.

TEST PRINCIPLE

The Green Spring® SARS-CoV-2 Antigen Rapid Test is a qualitative, membrane-based immunoassay for the detection of SARS-CoV-2 nucleocapsid protein antigen. The test line area is coated with SARS-CoV-2 antibody. The sample reacts with the SARS-CoV-2 antibody in the test line area. If the sample contains SARS-CoV-2 antigen, a coloured line appears in the test line area as a relevant result. As a procedural control, a coloured line appears in the control line area, indicating that the correct volume of sample has been applied and membrane wetting has proceeded correctly.

STORAGE AND STABILITY

Store the tests in the sealed foil pouch at room temperature or refrigerated (2 - 30 °C). The test is stable until the expiry date printed on it. The test cassettes must be stored in the sealed foil pouch until use. Do not freeze. Do not use after the expiry date. Protect from sun, moisture and heat.

MATERIALS SUPPLIED

- Test cassettes (25 pieces)
- Sampling swabs: 25 pieces
- Extraction tubes with buffer: 25 pieces disposable reaction tubes with 0.5 ml extraction buffer each and 25 pieces nozzle cap
- Package leaflet: 1 instruction leaflet
- Workstation: 1 piece
- Desiccant: 1 package

PRECAUTIONS

1. Read the package leaflet carefully before performing the test. Failure to follow the instructions in the package leaflet may result in inaccurate test results.
2. For professional in vitro diagnostic use only. Do not use after the expiry date.
3. Do not eat, drink or smoke for 10 minutes before and during sample collection.
4. Do not use the test if the packaging or test components are damaged.
5. All samples must be considered potentially infectious. Observe established precautions against microbiological hazards throughout the collection, handling, storage and disposal of patient samples and used test components.
6. Wear protective clothing such as lab coats, disposable gloves and eye protection while testing samples.
7. Wash your hands thoroughly after performing the test.
8. Viral transport media (VTM) may affect the test result: Extracted samples for PCR testing cannot be used for testing.
9. All used test components should be disposed of according to local regulations.
10. Humidity and temperature may adversely affect the results.

PREPARATION

Use only the materials supplied with the respective set. Test the samples immediately.

Use the test kit only at room temperature (15 to 30 °C). The test kit is intended only for swab samples that are collected and tested directly (i.e. swabs that have NOT been placed in transport media). This kit is NOT intended for testing liquid samples such as wash or aspirate samples or swabs in transport media, as results may be affected by over-dilution.

1. Tear off the foil pouch, take out the test cassette and place it on a clean and flat surface.
2. Freshly collected samples should be processed within 1 hour.
3. Label the respective test cassette for each test or control.
4. Place the labelled extraction tubes in a rack in the designated area of the workspace.

COLLECTING THE SAMPLE

Correct sample collection is the most important step. Select one of the four methods and then proceed with the test procedure.

1) Saliva (lollipop)
Be aware that false results may occur if the saliva is not collected properly.

1. Place an extraction tube in the cardboard workstation.

2. Press the tip of your tongue against the lower root of your jaw. Cough deeply. Make the sound of "kuuua" to gather the saliva.
3. Place the swab on the tongue for at least 10 seconds, rotating it 3 times or more to fully absorb the saliva.

2) Anterio-nasal swab (nose in front).
Make sure to collect enough nasal secretions with the swab. It is advisable to blow your nose first.

1. Place an extraction tube in the cardboard workstation.
2. Carefully insert the swab into the patient's nostril. The tip of the swab should be inserted up to 2.5 cm deep from the edge of the nostril.
3. Swab along the mucosa in the nostril to ensure that both mucus and cells are collected.
4. Take the swab out of the nostril while gently rotating it between your fingers.

3) Nasopharyngeal swab (nose-throat).

1. Place an extraction tube in the cardboard workstation.
2. Tilt the patient's head slightly backwards. Hold the swab like a pen and insert it through the nostril parallel to the palate.
3. While inserting, gently rub and roll the swab. As soon as you feel the throat resistance, stop and let the swab absorb secretion.
4. Slowly and carefully move the swab outwards while gently rotating it between your fingers.

4) Oropharyngeal swab (throat).

1. Place an extraction tube in the cardboard workstation.
2. Let the patient open the mouth wide and make "Ah" sounds, which will expose the pharyngeal tonsils on both sides.
3. Hold the swab tightly and wipe back and forth on the pharyngeal tonsils on both sides at least three times per side with moderate force. Do not touch the palate, tongue, teeth or gums.
4. Remove the swab while gently rotating it between your fingers.

For best results, the nasopharyngeal (nose-throat) method is recommended.

PERFORMING THE TEST

After collecting the sample, perform the test as follows:

1. Tear off the sealing of the extraction tube.
2. Insert the swab sample into the extraction tube and dip it up and down in the liquid. Rotate the swab several times during this process.
3. While removing the swab squeeze the sides of the tube to extract the remaining liquid our of the swab.
   Place the dropper tip firmly on the extraction tube and mix the liquid thoroughly .
4. Dispense 3 drops (approximately 100uL) into the sample well of the test cassette via the dropper tip.
5. Interpret the test results after 15 minutes. Do not interpret the results after 20 minutes.

INTERPRETING THE TEST RESULT

POSITIVE: Two lines appear. One coloured line appears in the control line area (C) and another coloured line appears in the test line area (T). A positive result in the test area indicates the detection of SARS-CoV-2 antigen in the sample. A positive result does not exclude infection with other pathogens.

NEGATIVE: A coloured line appears in the control area (C). No coloured line appears in the test line area (T). A negative result does not exclude viral infection with SARS-CoV-2 and should be confirmed by molecular diagnostic methods if COVID-19 is suspected.

INVALID: The control line does not appear. Insufficient sample volume or incorrect handling are the most likely reasons causing the control line not to appear. Check the procedure and repeat the test with a new test cassette. If the problem persists, stop using the test kit immediately and contact your dealer.

QUALITY CONTROL

The control area (C) acts as an internal procedure control. A coloured line appears when the procedure or sample volume has been applied correctly. Control standards are not provided with this test. As good laboratory practice, it is recommended to perform positive and negative controls periodically to verify test performance.

LIMITATIONS

This test is intended for the qualitative detection of SARS-CoV-2 virus antigen only. The exact concentration of SARS-CoV-2 viral antigen cannot be determined by this test.

Test results are for clinical reference only and should not be the sole basis for clinical diagnosis and treatment. Clinical management of patients should be considered in combination with their symptoms, physical signs, patient history, other laboratory tests, therapeutic responses and epidemiological information.

Proper sampling is crucial. Failure to follow the procedure can lead to incorrect test results. Improper collection, storage or even freezing and thawing of the sample can lead to inaccurate test results.

A false-negative test result may occur if the viral antigen level in a sample is below the detection limit of the test or if the sample was not collected or transported properly; therefore, a negative test result does not exclude the possibility of SARS-CoV-2 infection.

A positive result does not exclude co-infection with other pathogens.

Monoclonal antibodies may not detect SARS-CoV-2 viruses with slightly altered amino acid levels in the region of the target epitope, or may detect them with less sensitivity.

The amount of antigen in a sample may decrease with increasing disease duration. Samples collected after day 5 of illness are more likely to be negative compared to an RTPCR test.

The tests target the nucleocapsid proteins. Performance is not affected by mutations in the spike protein. Mutations in the nucleocapsid protein are not excluded in the future.

CHARACTERISTICS OF PERFORMANCE

The clinical performance of *the Green Spring® SARS-CoV-2 Antigen Rapid Test* was determined in prospective, randomised, single-blind studies. A total of 365 nasopharyngeal samples from symptomatic and asymptomatic patients were collected within 5 days of the onset of initial symptoms. The performance of the kit was compared with the results of a commercially available molecular test. The PCR comparisons use a nasopharyngeal swab.

Table 1: Clinical study nasopharyngeal (nose-throat)

Green Spring SARS-COV-2 Antigen Rapid Test	**PCR-Comparison**		**Total**
	Positive	**Negative**	
Positive	150	0	150
Negative	5	210	215
Total	155	210	365
Sensitivity	**96,77%** (95%KI: 92,24-98,81%)		
Specificity	**100,00%** (95%KI: 97,76-100%)		
Accuracy	**98,63%** (95%KI: 96,89-100%)		

PPA(Ct≤ 37): 96,77% (150/155), (95%KI: 92,24-98,81%)
NPA(Ct≤ 37): 100,00% (210/210), (95%KI: 97,76-100%)

For the anterior nasal swab method, a total of 298 anterior nasal samples were collected from symptomatic and asymptomatic patients within 5 days of the onset of the first symptoms. The performance of

the kit was compared with the results of a commercially available molecular test. The PCR comparisons use a nasopharyngeal swab.

Table 2: clinical study anterior-nasal (nose-front)

Green Spring SARS-COV2 Antigen Rapid Test	**PCR-Comparison**		**Total**
	Positive	**Negative**	
Positive	154	0	154
Negative	6	138	144
Total	160	138	298
Sensitivity	**96,25%** (95%KI: 91,65-98,47%)		
Specificity	**100,00%** (95%KI: 96,62-100%)		
Accuracy	**97,99%** (95%KI: 96,97-100%)		

PPA(Ct≤ 37): 96,25% (154/160), (95%KI: 91,65-98,47%)
NPA(Ct≤ 37): 100,00% (138/138), (95%KI: 96,62-100%)

For the saliva swab method, a total of 298 saliva samples from symptomatic and asymptomatic patients were collected within 5 days of the onset of the first symptoms. The performance of the kit was compared with the results of a commercially available molecular test. The PCR comparisons use a nasopharyngeal swab.

Table 3: clinical study saliva (lollipop)

Greenspring SARS-COV2 Antigen Rapid Test	**PCR-Comparison**		**Total**
	Positive	**Negative**	
Positive	147	0	147
Negative	13	138	151
Total	160	138	298
Sensitivity	**91,88%** (95%KI: 86,22-95,43%)		
Specificity	**100,00%** (95%KI: 96,62-100%)		
Accuracy	**95,64%** (95%KI: 93,32-97,96%)		

PPA(Ct≤ 37): 91,88% (147/160), (95%KI: 86,22-95,43%)
NPA(Ct≤ 37): 100,00% (138/138), (95%KI: 96,62-100%)

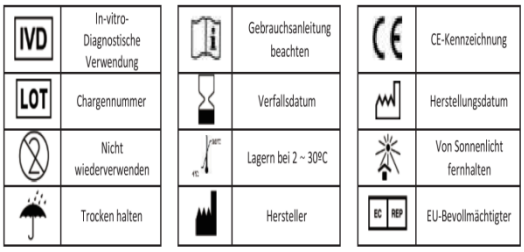
CROSS-REACTIVITY

Potential cross-reactant	**Concentration**	**Cross-reactivity (Yes/No)**
Influenza A	1.6 x 10^5 $TCID_{50}$/mL	No
Influenza B	1.6 x $10^5$$TCID_{50}$/mL	No
Human coronavirus HKU1	1.6 x 10^5 $TCID_{50}$/mL	No
Human coronavirus OC43	1.6 x 10^5 $TCID_{50}$/mL	No
Haemophilus influenzae	2.2x 10^5 $TCID_{50}$/mL	No
MERS-coronavirus	2.1 x 10^5 $TCID_{50}$/mL	No
SARS-coronavirus	3.2 x 10^5 PFU/mL	Yes
Adenovirus C1	1.5 x 10^5 $TCID_{50}$/mL	No
Adenovirus 71	1.5 x 10^5 $TCID_{50}$/mL	No
Candida albicans	4.2 x 10^5 CFU/mL	No
Respiratory syncytial virus	5.1 x 10^5 $TCID_{50}$/mL	No
Enterovirus	5.4 x 10^5 $TCID_{50}$/mL	No
Malaria	2.2 x 10^6 CFU/mL	No
Dengue	1.2 x 10^5 $TCID_{50}$/mL	No
Human coronavirus NL63	1.7x 10^5 $TCID_{50}$/mL	No
Human coronavirus 229E	2.2 x 10^5 $TCID_{50}$/mL	No
Streptococcus pneumoniae	1.1 x 10^6CFU/mL	No
Pneumocystis jirovecii (PJP)	1.0 x 10^5 $TCID_{50}$/mL	No
Legionella pneumophila	1.4 x 10^6 CFU/mL	No
Chlamydia pneumoniae	1.1 x 10^6 IFU/mL	No
HumanMetapneumovirus(hMPV)	1.1 x 10^5 $TCID_{50}$/mL	No
Parainfluenza virus 1	1.0 x 10^5 $TCID_{50}$/mL	No
Parainfluenza virus 2	1.0 x 10^5 $TCID_{50}$/mL	No
Parainfluenza virus 3	3.5 x 10^5 $TCID_{50}$/mL	No
Parainfluenza virus 4	1.4 x 10^5 $TCID_{50}$/mL	No
Rhinovirus	1.3 x 10^5 PFU/mL	No
Mycoplasma pneumoniae	1.8 x 10^6 CFU/mL	No
Bordetella pertussis	1.5 x 10^6 CFU/mL	No
Mycobacterium tuberculosis	1.0 x 10^6 CFU/mL	No
Concentrated human nasal contents (representative of normal respiratory microbial flora)	100%	No
Streptococcus pyogenes	1.0 x 10^6 CFU/mL	No

INTERFERENCE

SARS-CoV-2 antigen nasal swab samples were mixed with one of the following substances to specific concentrations and tested in multiple replicates. No false-positives or false-negatives were found:

Substance	**Concen-tration**	**Substance**	**Concen-tration**
Whole Blood	5%	Naso GEL(Nei Med)	6%v/v
Fluticasone Propionate	4%v/v	Mucin	0.54%
CVS Nasal Drops(Phenylephrine)	17%v/v	Ricola(Menthol)	1.6mg/mL
Tamiflu (Oseltamivir Phosphate)	6mg/ml	Afrin (Oxymetazoline)	14%v/v
Sucrets (Dyclonin/Menthol)	1.4 mg/mL	CVC Nasal Spray(Cromolyn)	16%v/v
Chloraseptic (Menthol/Benzocaine)	1.8 mg/mL	Nasal Gel (Oxymetazoline)	9%v/v
Homeopathic(Alkalol)	1:10dilution	Mupirocin	12 mg/mL
Ore Throat Phenol Spray	16%v/v	Fisherman's Friend	1.3mg/mL
Tobramycin	5 μg/mL	Zicam	4%v/v



In-vitro diagnostic use	Read the instruction before using	CE marking
Batch number	Expiry date	Date of manufacture
Do not re-use it	Store at 2°C-30°C	Keep away from sunlight
Keep dry	Manufacturer	EU authorised representative

LIMIT OF DETECTION (ANALYTICAL SENSITIVITY)

The limit of detection (LOD) for the *Green Spring® SARS-CoV-2 Antigen Rapid Test* is 4 x 10^2 $TCID_{50}$/mL. The LOD for Green Spring® SARS-CoV-2 Antigen Rapid Test Kit was determined using limiting dilution of a gamma irradiation inactivated virus sample. The sample was provided at a concentration of 1.3×10^6 $TCID_{50}$/mL.

HIGH-DOSE HOOK EFFECT

The LOD study tested the highest concentration of the sample ($TCID_{50}$ of 1.3 x 10^6 $TCID_{50}$/mL). No hook effect was observed.

FURTHER PRODUCT INFORMATION

Manufacturer: Shenzhen Lvshiyuan Biotechnology Co., Ltd

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