



Shenzhen Lvshiyuan Biotechnology Co.,Ltd



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

REF GF102B1 Rev. 7

English

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

INTENDED USE

The Green Spring® SARS-CoV-2 Antigen Rapid Test Kit is for the rapid qualitative detection of SARS-CoV-2 nucleocapsid protein antigen in human saliva, nasal, nasopharyngeal or oropharyngeal swab specimens. The results are used for the detection of SARS-CoV-2 antigens. The antigen is generally detectable in upper respiratory tract specimens during the acute phase of infections. Positive results do not rule out bacterial infection or co-infection with other viruses. The pathogen detected may not be the sole cause of the disease.

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. Suspected cases should be confirmed with a molecular assay. For professional use only.

SUMMARY

The novel coronaviruses belong to a β genus. COVID-19 is an acute respiratory infectious disease. Humans are generally susceptible to it. Currently, patients infected with novel coronavirus are the main source of infection; asymptomatic infected people may also be a source of infection. The main manifestations include fever, fatigue, and dry cough. A stuffy or runny nose, sore throat, muscle aches, and diarrhea 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 nucleocapsid protein antigens of SARS-CoV-2. The test line region is coated with a SARS-CoV-2 antibody. The sample reacts with the SARS-CoV-2 antibody in the test line region. If the specimen contains SARS-CoV-2 antigens, a colored line appears in the test line region (T) as a result. As a procedural control, a colored line appears in the control line region (C) indicating that the correct volume of sample has been added and membrane wicking has occurred 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. The test cassettes should be kept in the sealed foil pouch until use. Do not freeze. Do not use after expiration date. Keep away from sun, moisture and heat.

MATERIALS SUPPLIED

- Test cassette with a pack of desiccant: 25 pieces
- Sterile swab: 25 pieces
- Extraction tube with buffer and nozzle cap: 25 pieces disposable extraction tubes with 0.5 ml extraction buffer each
- Tube rack: 1 piece
- Package insert: 1 instruction manual

WARNINGS AND PRECAUTIONS

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

PREPARATION

Only use the materials supplied with the set. Test the specimens immediately.

Let the test kit equilibrate to room temperature (15 to 30 °C). The test kit is intended only for swab specimens 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 specimens such as wash or aspiration samples or swabs in transport media, as results may be affected by over-dilution.

1. Tear off the foil pouch, remove 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 or control for each test.
4. Place the labeled extraction tubes in a rack in the designated area of the work area.

SPECIMEN COLLECTION

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

1) Saliva (Lolly-Test)

Be aware that incorrect results may occur if the saliva is not collected properly.

1. Place an extraction tube in the cardboard tube rack.
2. Press the tip of the tongue against the lower root of the jaw. Cough deeply. Make the sound of "kuuuu" to concentrate the saliva.
3. Place the swab on the tongue for at least 10 seconds, rotate it 3 times or more to fully absorb the saliva.

2) Anterior-nasal swab

Be sure to collect sufficient nasal secretions with the swab. It is recommended to blow your nose first.

1. Place an extraction tube in the cardboard tube rack.
2. Carefully insert the swab into the patient's nostril. The swab tip 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. Remove the swab from the nostril while gently rotating it between your fingers.

3) Nasopharyngeal swab

1. Place an extraction tube in the cardboard tube rack.
2. Tilt the patient's head slightly backward. 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. Once you feel pharyngeal resistance, stop and allow the swab to absorb secretions.
4. Slowly and gently remove the swab outward while gently rotating it between your fingers.

4) Oropharyngeal swab

1. Place an extraction tube in the cardboard tube rack.
2. Ask the patients open their mouth wide and make "Ah" sounds, exposing the pharyngeal tonsils on both sides.
3. Hold the swab firmly 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 method is recommended.

TEST PROCEDURE

After taking the sample, perform the test as follows:

1. Tear off the seal of extraction buffer tube.
2. Insert the swab into the tube and dip the swab up and down in the liquid for at least 10 seconds. Then, hold the swab against the bottom of the tube and rotate it 3 turns, making sure that no contents splash out of the tube.
3. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab.
4. Place the dropper tip firmly on the extraction buffer tube and mix the liquid thoroughly.
5. Dispense 3 drops (approximately 100µL) into the sample well of the test cassette via the dropper tip.
6. Interpret the test results after 15 minutes. Do not interpret the results after 20 minutes.

INTERPRETATION OF THE TEST RESULT

POSITIVE: Two lines appear. One colored line appears in the control line region (C) and another colored line appears in the test line region (T). A positive result in the test region indicates the detection of SARS-CoV-2 antigens in the specimen but does not rule out infection with other pathogens.

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

INVALID: Control line does not appear. Insufficient sample volume or incorrect handling are the most likely reasons for the control line not appearing. 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 distributor.

QUALITY CONTROL

The control region (C) serves as an internal procedure control. A colored 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 that positive and negative controls be performed periodically to verify test performance.

LIMITATIONS

- This test is for the qualitative detection of SARS-CoV-2 Virus antigens only. The exact concentration of SARS-CoV-2 virus antigens 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 epidemiologic information.
- Proper specimen collection is critical. Failure to follow the procedure can lead to inaccurate test results. Improper collection, storage, or even freezing and thawing of the specimen can lead to inaccurate test results.
- A false-negative test result may occur if the viral antigen level in a specimen is below the detection limit of the test or if the specimen was not collected or transported properly; therefore, a negative test result does not rule out the possibility of SARS-CoV-2 infection.
- A positive result does not rule out 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 RT-PCR test.
- The test target is the nucleocapsid proteins. Performance is not affected by mutations in the spike protein. Mutations in the nucleocapsid protein are not excluded in the future.

CLINICAL PERFORMANCE

The clinical performance of the Green Spring® SARS-CoV-2 Antigen Rapid Test was determined in prospective, randomized, single-blind studies. A total of 365 nasopharyngeal specimens 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)

Green Spring SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold)	PCR-Comparator		Total
	Positive	Negative	
Positive	150	0	150

Negative	5	210	215
Total	155	210	365
Sensitivity	96,77% (95%CI: 92,24-98,81%)		
Specificity	100,00% (95%CI: 97,76-100%)		
Accuracy	98,63% (95%CI: 96,89-100%)		

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

For the anterior nasal swab method, a total of 298 anterior nasal specimens 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 assay. The PCR comparisons use a nasopharyngeal swab.

Table 2: clinical study (anterior-nasal)

Green Spring SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold)	PCR-Comparator		Total
	Positive	Negative	
Positive	154	0	154
Negative	6	138	144
Total	160	138	298
Sensitivity	96,25% (95%CI: 91,65-98,47%)		
Specificity	100,00% (95%CI: 96,62-100%)		
Accuracy	97,99% (95%CI: 96,97-100%)		

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

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

Table 3: clinical study (Saliva)

Green Spring SARS-CoV-2 Antigen Rapid Test Kit (Colloidal Gold)	PCR-Comparator		Total
	Positive	Negative	
Positive	147	0	147
Negative	13	138	151
Total	160	138	298
Sensitivity	91,88% (95%CI: 86,22-95,43%)		
Specificity	100,00% (95%CI: 96,62-100%)		
Accuracy	95,64% (95%CI: 93,32-97,96%)		

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

CROSS-REACTIVITY

No cross-reactivity with potentially cross-reactive agents was observed, other than SARS coronavirus.

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	2.2 x 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	1.7 x 10 ⁵ TCID ₅₀ /mL	No
Human coronavirus 229E	2.2 x 10 ⁵ TCID ₅₀ /mL	No
Streptococcus pneumoniae	1.1 x 10 ⁶ CFU/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
Human Metapneumovirus(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
Konzentrierte menschliche Naseninhalte repräsentativ für normale respiratorische mikrobielle Flora	100%	No
Streptococcus pyogenes	1.0 x 10 ⁶ CFU/mL	No

INTERFERENCE

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

Substance	Concentration	Substance	Concentration
Whole Blood	5%	Naso GEL(Nei Med)	6%v/v
Fluticasone	4%v/v	Mucin	0,54%

Propionate			
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

Limit of Detection (analytical sensitivity)

The limit of detection (LOD) for the Green Spring ® SARS-CoV-2 Antigen Rapid Test is 4×10^2 TCID₅₀/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 $1,3 \times 10^6$ TCID₅₀/mL.

HIGH-DOSE HOOK-EFFECT

The LOD study tested the highest concentration of the sample (TCID₅₀ von $1,3 \times 10^6$ TCID₅₀/mL). No Hook-effect was detected.

FURTHER PRODUCT INFORMATION

Manufacturer: Shenzhen Lvshiyuan Biotechnology Co., Ltd

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SYMBOL DESCRIPTION

IVD	In Vitro Diagnostic Use	Instruction for Use	CE Mark
LOT	Batch Number	Expiry Date	Manufacturing Date
Do not reuse		Store between 2~30°C	Keep away from Sunlight
Keep Dry		Manufacturer	EU Authorized Representative