BNG® SARS-CoV-2 Antigen Rapid Test

Self Test Instructions for Use

For use with anterior nasal swab specimens

For in vitro Diagnostic Use Only

INTENDED USE

BNG® SARS-CoV-2 Antigen Rapid Test is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2.

This test is authorized for non-prescription home use with self-collected anterior nasal swab samples from individuals aged 15 years or older with symptoms of COVID-19 within the first seven days of symptom onset. This test is also authorized for non-prescription home use with adult-collected anterior nasal swab samples from individuals aged two years or older with symptoms of COVID-19 within the first seven days of symptom onset.

This test is also authorized for non-prescription home use with self-collected anterior nasal swab samples from individuals aged 15 years or older, or adult-collected anterior nasal swab samples from individuals aged 2 years or older, with or without symptoms or other epidemiological reasons to suspect COVID-19 when tested twice over three days with at least 34 hours between tests.

The BNG® SARS-CoV-2 Antigen Rapid Test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of the SARS-CoV-2 nucleocapsid protein antigen. The antigen is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with past medical 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. Individuals who test positive with the BNG® SARS-CoV-2 Antigen Rapid Test should self-isolate and seek follow-up care with their physician or healthcare provider as additional testing may be necessary.

Negative results are presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed. Negative results do not rule out COVID-19 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 an individual's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

For serial testing programs, additional confirmatory testing with a molecular test for negative results may be necessary, if there is a high likelihood of COVID-19, such as, an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection. Additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in

individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection.

SUMMARY AND EXPLANATION OF THE TEST

The SARS-CoV-2 is an enveloped β-coronavirus, circular or elliptical particle diameter of about 60 ~ 140nm, often pleomorphic, obviously different from SARS- CoV and MERS-CoV in genetic characteristics. The main clinical manifestations include fever, fatigue and other systemic symptoms, accompanied by dry cough, dyspnea, etc., which can rapidly develop into severe pneumonia, respiratory failure, acute respiratory distress syndrome, septic shock, multi-organ failure, severe acid-base metabolism disorder, and even life-threatening. SARS-CoV-2 has been identified as the main means of transmission through respiratory droplets (sneezing, coughing, etc.) and contact (picking nostril with the hand in contact with the virus, rubbing eyes, etc.).

SARS-CoV-2 is sensitive to ultraviolet ray and heat, and can be inactivated at 56℃ for 30 minutes and by fat soluble solvent such as ethyl ether, 75% ethanol, chlorine disinfectant, peracetic acid and chloroform.

The BNG® SARS-CoV-2 Antigen Rapid Test is a rapid lateral flow immunoassay for the qualitative detection of SARS-CoV-2 directly from anterior nasal swabs, The BNG® SARS-CoV-2 Antigen Rapid Test kit contains all components required to carry out an assay for SARS-CoV-2.

PRINCIPLE OF PROCEDURES

BNG® SARS-CoV-2 Antigen Rapid Test employs immuno-lateral chromatography technology for the qualitative detection of antigens. The colloidal gold particles labeled with the anti-SARS-CoV-2 antibody 1 are fixed on the conjugation pad. The anti-SARS-CoV-2 antibody 2 is bound on the “T” test line of nitrocellulose membrane. The Goat Anti-Mouse IgG is bound on the “C” control line of nitrocellulose membrane. When the concentration of SARS-CoV-2 in the specimen is higher than the minimum detection limit, which can conjugate with the anti-SARS-CoV-2 antibody 1 labeled with colloidal gold particles to form a complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the anti-SARS-CoV-2 antibody 2 bound on the test line, forming “Au-Anti-SARS-CoV-2 antibody 1-(SARS-CoV-2) - Anti-SARS-CoV-2 antibody 2 complex. These complexes are deposited to display color as the determination of antigen positive, the rest of anti-SARS-CoV-2 antibody 1 labeled with colloidal gold particles conjugate with the Goat Anti-Mouse IgG and deposit to display color as the determination of quality of the “C” control line. When the concentration of SARS-CoV-2 in the specimen is lower than the minimum detection limit or no SARS-CoV-2, the complexes only deposit and display color in the “C” control line.

REAGENTS and MATERIALS

1 test/kit, 5 tests/kit, 10 tests/kit, 20 tests/kit, 25 tests/kit, Test device: Mouse anti- SARS-CoV-2 monoclonal antibody, Goat Anti-Mouse IgG polyclonal antibody, Nitrocellulose membrane.

- Extraction solution: Phosphate Buffer solution (0.01M, pH7.4±0.2)
- 1 Extraction tube
- 1 Package insert (Instructions for Use)
- 1 Disposable swabs (Anterior nasal swab)

MATERIALS REQUIRED BUT NOT PROVIDED

Timer

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

• This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.

• This test is intended as an aid in the diagnosis of COVID-19 by detecting viral antigens, but should not be used as a sole criterion for the determination of SARS-CoV-2 infection. Other laboratory tests and clinical information (signs and symptoms) should be used and considered for diagnosis.

• Do not use any test component after the expiration date which is printed on the outer packaging.

• Do not use the COVID-19 Test Card if the pouch is damaged or if the seal is broken.

• All kit components are single-use items. Do not use with multiple specimens. Do not reuse the used test card or swab.

• To obtain accurate results, the test must be performed as indicated in the Instructions for Use.

• Inadequate or inappropriate sample collection may yield false test results.

• Do not touch the tip of the swab before and after collecting the sample from the nostrils.

• Test samples immediately after collection, but no more than 4 hours after specimen collection before placement into extraction buffer or up to 2 hours after placement into extraction buffer, if kept at room temperature.

• Be sure to read test result after 15 minutes. Do not read results after 30 minutes.

• Do not ingest extraction liquid

• Keep test kit and components out of the reach of children and pets before and after use.

• Avoid contact with skin and eyes.

• The Reagent Solution contains a harmful chemical. If the solution contacts the skin or eye, flush with copious amounts of water. If skin irritation or rash occurs get medical advice/attention.

STORAGE and STABILITY

Store the BNG® SARS-CoV-2 Antigen Rapid Test in a dry location between 35.6-86°F (2-30°C), out of direct sunlight, valid for 12 months. Do not freeze the kit or its components. The test device should be used within 60 minutes after opening the foil bag. For the production date and expiration date, please refer to the product label.

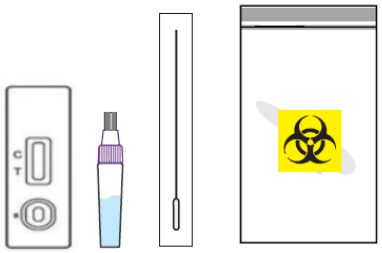
SPECIMEN REQUIREMENTS

The BNG® SARS-CoV-2 Antigen Rapid Test is for prescription use at home and other non-laboratory sites and/or over the counter (OTC) use at home and other non-laboratory sites. It is important to read the instruction for use carefully and follow the steps in the correct order. It takes about 15 minutes to prepare before each test, and the results can be obtained after 30 minutes. Please use the test kit at room temperature (15-30 °C). If the test kit was previously stored in a cool place (temperature below 15 °C), please balanced at 15-30 °C for 30 minutes before test.

Before Starting

*Wash hands thoroughly with soap water/ hand sanitizer. Make sure they are dry before starting. This step ensures that the kit is not contaminated.

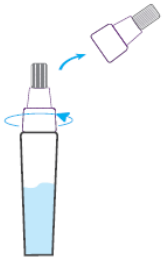
*Check the components in the box and verify that there is not any damage or breakage.



Sample Collecting

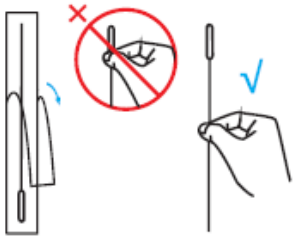
1. Remove the Swab, Extraction Tube, and Test Device from their packaging. Please keep the package for later use.

2- Twist the large cap to open the extraction tube.

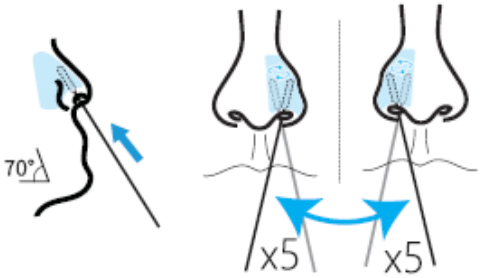


3-Tear off the swab's packing from the stick end and take out the swab.

! Keep fingers away from the swab end.



4-Gently insert swab soft head into nostril less than 1 inch (Usually 1/2 or 3/4 of an inch). Gently rotate the swab against the nasal wall with moderate force at least five times. Firmly sample the nasal wall by rotating the swab in a circular path against the nasal wall at least 5 times. Take at least 15 seconds to collect the specimen and be sure to collect any nasal drainage on the swab. Repeat in the other nostril using the same swab.



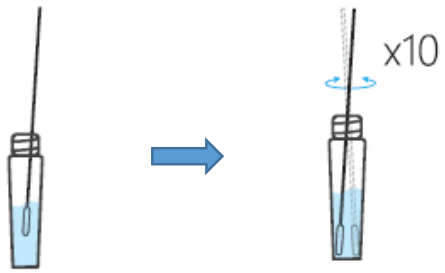
!Do not insert too far into the nostril as this may lead to nasal cavity bleeding or swab rupture and other risks.

!False-negative results may occur if the nasal swab is not properly collected.

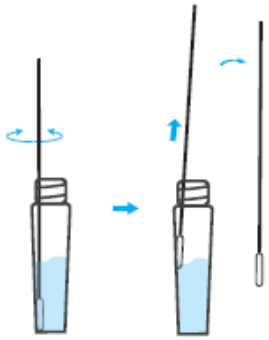
!The person under 18 years of age should be tested with the assistance of the legal guardian or authorized person.

Sample Processing

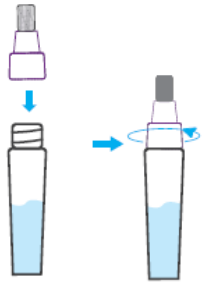
1-Dip the swab head into the extraction solution in the extraction tube and rotate the swab close to the specimen extraction tube wall for about 10 seconds or 10 times to dissolve the specimens in the solution as much as possible.



2-Squeeze the tip of the swab along the inner wall of the specimen extraction tube to keep the liquid in the tube as much as possible, take out the swab.



3-Tighten the tube cap and wait. Then, twist to open the small cap of the tube.

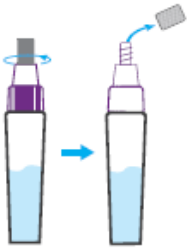


Sample Testing

1- Tear off the aluminum foil bag, take out the test card and place horizontally on the test desk.

! The platform should be in a horizontal and stable state, and tilt and shake are strictly prohibited.

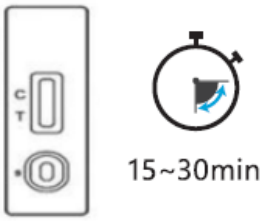
2-Twist to open the small white cap of the tube.



3-Gently, squeeze the extraction tube, and drop 2 drops of liquid vertically into the sample well (S)of the test card.

! The existence of the bubbles in the extraction tube may lead to the wrong sample volume and inaccurate test results. If there are bubbles in the extraction tube, gently shake the extraction tube to squeeze out part of the liquid so as to remove the bubbles.

4-Start timing, read the results at 15 minutes. Do not read results before 15 minutes or after 30 minutes.



5- After test is completed, put all the test kit materials into the biohazard waste bag and dispose it according to the local biohazard waste disposable policy.

INTERPRETATION OF TEST RESULTS

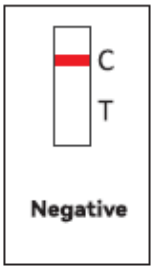
***Positive Result**

A POSITIVE result must show both a C line and a T line. A colored one Line appears in the control area (C) and another colored line appears in the test area (T) regardless of the strength of the test line. A positive result in the test region indicates the detection of SARS-COV-2 antigens in the sample.

Please note that the T line may be faint.

*** Negative Result**

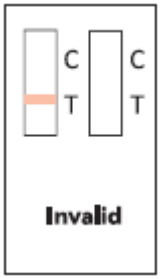
A NEGATIVE result will show only a C line. No colored line appears in the test line region (T). A negative result indicates that viral antigens from COVID-19 were not detected.



*** Invalid Result**

The control line is not displayed. Insufficient sample volume or a wrong procedure/ Handling are the most likely reasons for not Appearance of the control line. Check the procedure and repeat that Test with a new test kit. If the problem persists, exit the use the batch immediately, and contact your local dealer.

Note: The color of the test strip will vary with different samples. However, regardless of the color of the test strip, it should be judged as a positive result within the specified detection time.



LIMITATION

1. The BNG® SARS-CoV-2 Antigen Rapid Test is for in vitro diagnostic use only. The test should be used for the detection of SARS-CoV-2 Antigen in anterior nasal swab specimens only.
2. This test kit can only be used for the qualitative detection of SARS-CoV-2 antigens and can't determine the quantity of SARS-CoV-2 antigens in samples.
3. If the test result is negative and clinical symptoms persist. It is recommended to repeat sampling or use other testing methods for testing. A negative result cannot preclude the possibility of exposure to or infection with SARS-CoV-2 virus at any time.

4. The test results of the test kits are for clinicians' reference only and should not be used as the only basis for clinical diagnosis. The clinical management of patients should be comprehensively considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment responses, etc.
5. Due to the limitation of the detection reagent methodology, the limit of detection of this reagent is generally lower than that of nucleic acid reagents. Therefore, the test personnel should pay more attention to the negative results and need to combine other test results to make a comprehensive judgment. It is recommended to use nucleic acid testing or virus isolation and culture identification methods to review negative results which have doubts.
6. Analysis of the possibility of false negative results:
   1. Unreasonable specimen collection, transportation and processing, low virus titer in the sample, no fresh sample or freezing and thawing cycling of the sample may lead to false-negative results.
   2. The mutation of viral gene may lead to changes in antigenic determinants, which lead to negative results.
   3. The research on the SARS-CoV-2 has not been completely thorough; the virus may mutate and cause differences for best sampling time (virus titer peak) and sampling location. Therefore, for the same patient, we can collect samples from multiple locations or follow up for multiple times reduce the possibility of false-negative results.

PERFORMANCE CHARACTERISTICS

Clinical Performance

Clinical performance characteristics of the BNG® SARS-CoV-2 Antigen Rapid Test were evaluated in a total of two investigational sites. A total of 420 individuals with signs and symptoms of COVID-19 within the first seven (7) days of symptom onset completed the study and obtained a valid result. Each Subject was provided a BNG® SARS-CoV-2 Antigen Rapid Test Under the observation of a clinical site staff member trained as a proctor, subjects fifteen (15) years and older independently collected an anterior nasal sample, conducted the test, interpreted and reported their self-test result. The parents of subjects two (2) to fourteen (14) years of age collected the anterior nasal sample, conducted the test, interpreted, and recorded the test result for the child. An FDA Emergency Use Authorized real-time Polymerase Chain Reaction (RT-PCR) assay for the detection of SARS-CoV-2 was utilized as the comparator method for this study. The BNG® SARS-CoV-2 Antigen Rapid Test when conducted by a lay user correctly identified 97.55% of positive samples. Additionally, the BNG® SARS-CoV-2 Antigen Rapid Test correctly identified 100% of negative samples.

The test results of BNG® SARS-CoV-2 Antigen Rapid Test and the PCR test results are shown in the following table:

BNG® SARS-CoV-2 Antigen Rapid Test	**Comparator Method**		
	Positive (+)	**Negative (-)**	**Total**
Positive (+)	159	0	159
Negative (-)	4	257	261
Total	163	257	420
Positive Agreement: 159/163 97.55% (95% CI: 93.84% to 99.33%)			
Negative Agreement: 257/257 100.0% (95% CI: 98.57% to 100.00%)			

Patient demographics, time from symptom onset for all patients participating in the above study, are presented in the table below.

Positive Results by days since symptom onset			
Days Since Symptom Onset	RT-PCR Positive (+)	BNG® Test Positive (+)	PPA
1	24	24	100.0%
2	30	28	93.3%
3	55	55	100.0%
4	72	69	95.8%
5	104	102	98.1%
6	133	133	100.0%
7	163	159	%97.54

Limit Of Detection

Positive value/limit of detection: 1.7×10^2 $TCID_{50}$/mL

Select the confirmed inactivated SARS-CoV-2 medium, (concentration $10^{7.5}$ $TCID_{50}$/mL), use gradient dilution method to find out the virus medium to reach the critical value of the detection. Repeat the action for 20 time and the test result is positive for at least 19 times.

Cross-Reactivity (Analytical Specificity) And Microbial Interference

The potential cross-reactivity (exclusivity) of a panel of common organisms was evaluated with SARS-CoV-2 negative samples using the BNG SARS-CoV-2 Antigen Rapid Test. A total of 33 commensal and pathogenic microorganisms that may be present in the nasal and throat cavity were evaluated in this study. Each of the organisms and viruses was tested in five replicates in the absence or presence of heat-inactivated SARS-CoV-2 virus. No cross-reactivity or interference was observed with the following microorganisms when tested at the concentration presented in the table below.

Name	Concentration	Test result
Influenza B/Y amagata	1.00×10^2 $TCID_{50}$/mL	Negative
Influenza A H3N2	1.15×10^2 $TCID_{50}$/mL	Negative
Adenovirus 3	1.24×10^5 $TCID_{50}$/mL	Negative
Adenovirus 7	1.87×10^6 $TCID_{50}$/mL	Negative
Human coronavirus 229E	1.00×10^5 $TCID_{50}$/mL	Negative
Human coronavirus OC43	2.00×10^6 $TCID_{50}$/mL	Negative
Human coronavirus NL63	2.00×10^6 $TCID_{50}$/mL	Negative
Human Metapneumovirus (hMPV)	1.00×10^5 $TCID_{50}$/mL	Negative
MERS-coronavirus	2.00×10^6 $TCID_{50}$/mL	Negative
Cytomegalovirus	1.00×10^5 $TCID_{50}$/mL	Negative
Enterovirus 71	2.55×10^5 $TCID_{50}$/mL	Negative
Human parainfluenza virus 1	1.35×10^5 $TCID_{50}$/mL	Negative
Human parainfluenza virus 2	6.31×10^5 $TCID_{50}$/mL	Negative
Human parainfluenza virus 3	3.25×10^5 $TCID_{50}$/mL	Negative
Human parainfluenza virus 4	1.0×10^5 $TCID_{50}$/mL	Negative
Respiratory syncytial virus	2.00×10^5 $TCID_{50}$/mL	Negative
Rhinovirus 1A	1.26×10^5 $TCID_{50}$/mL	Negative
Chlamydia pneumoniae	1.00×10^5 CFU/mL	Negative
Haemophilus influenzae	1.20×10^6 CFU/mL	Negative

Mycobacterium tuberculosis	1.00×10^5 CFU/mL	Negative
Mycoplasma Pneumoniae	1.00×10^6 CFU/mL	Negative
Neisseria gonorrhoeae	1.00×10^5 CFU/mL	Negative
Pseudomonas aeruginosa	3.70×10^6 CFU/mL	Negative
Staphylococcus aureus	2.20×10^6 CFU/mL	Negative
Streptococcus pneumoniae	1.00×10^6 CFU/mL	Negative
Streptococcus pyogenes	1.28×10^6 CFU/mL	Negative
Streptococcus salivarius	1.00×10^5 CFU/mL	Negative
Staphylococcus epidermidis	1.00×10^6 CFU/mL	Negative
Bordetella pertussis	1.00×10^6 CFU/mL	Negative
Legionella pneumophila	1.00×10^6 CFU/mL	Negative
Pneumocystis jirovecii (PJP)	1.00×10^6 CFU/mL	Negative
Candida albicans	1.00×10^6 CFU/mL	Negative
Pooled human nasal wash – representative of normal respiratory microbial flora	N/A	Negative

An in-silico analysis was performed using the Basic Local Alignment Search Tool (BLASTp) managed by the National Center for Biotechnology Information (NCBI) for Human Coronavirus HKU1, Mycobacterium tuberculosis, Pneumocystis jirovecii and SARS-CoV-1

• Human Coronavirus HKU1 shows 36.74% homology across 82% of the nucleocapsid sequence(see Annex 2 and 3), which is relatively low. However, cross-reactivity cannot be ruled out.

• *Mycobacterium tuberculosis* shows no protein sequence homology with nucleocapsid sequence. Therefore, while cross-reactivity is highly unlikely, it cannot be completely ruled out.

• *Pneumocystis jirovecii* shows no protein sequence homology with nucleocapsid sequence. Therefore, while cross-reactivity is highly unlikely, it cannot be completely ruled out.

• SARS-CoV-1 shows 90.52% homology across 100% of the nucleocapsid sequence. Therefore, cross-reactivity is highly likely.

Endogenous Interfering Substances

The following substances, naturally present in respiratory specimens, were evaluated with the BNG SARS-CoV-2 Antigen Rapid Test.

All samples tested in 5 replicates produced expected results, demonstrating that the BNG SARS-CoV-2 Antigen Rapid Test performance was not affected by any of the 20 potentially interfering substances listed in the table below at the concentrations tested.

Interfering substance name	Concentration	Negative interference result	Positive interference result
Mucin	5%	Negative	Positive
Whole blood	5% (V/V)	Negative	Positive
Zanamivir	500ng/mL	Negative	Positive
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	Negative	Positive
Naso GEL (NeilMed)	5% v/v	Negative	Positive
CVS Nasal Spray (Cromolyn)	15% v/v	Negative	Positive
Afrin (Oxymetazoline)	15% v/v	Negative	Positive
Zicam	5% v/v	Negative	Positive
Homeopathic (Alkalol)	1:10 dilution	Negative	Positive
Sore Throat Phenol Spray	15% v/v	Negative	Positive
Mupirocin	10 mg/mL	Negative	Positive

Tamiflu (Oseltamivir Phosphate)	5 mg/mL	Negative	Positive
Cepacol Lozenges(benzocaine/menthol)	3 mg/mL	Negative	Positive
Chloroseptic Sore Throat spray (Phenol, Glycerin)	5% v/v	Negative	Positive
Naoacort Allergy 24 hours (Triamcinolone)	15% v/v	Negative	Positive
Tobramycin	4ng/mL	Negative	Positive
Phenylephrine	20µg/mL	Negative	Positive
Crest/Listerine Mouthwash (Eucalyptol, menthol, Methyl Salicylate, Thymol)	5% v/v	Negative	Positive
Flunisolide	0.1mg/mL	Negative	Positive
Sodium chloride	5%	Negative	Positive

High Dose Hook Effect

Within the concentration of $10^{7.5}$ $TCID_{50}$/mL for cell culture medium of SARS-CoV-2 antigen, the test results of this product showed no Hook effect.

Usability Study

Twenty-two individuals of the age between 15 and 79 years (60 females, 56 male) were selected for this usability study. The participants represent a wide range of age groups, background and different education levels. 38 layperson were at least university graduate, 20 were high school graduate, and three was high school student. The questionnaire addresses questions about the comprehension of the test procedure steps as well as the practical application of the test device. 53 questions were included that could be answered in four different grades from very easy/perfect, easy/understood, moderate/partially understood and difficult/need of improvement. All questions of the questionnaire were answered predominantly by the two highest grades (very easyleasy and perfect/understood). The illustrations in the instruction for use and the description for the interpretation of test results were rated once as 'easy'. As the sampling procedure (illustrations are included), testing procedure and results interpretations are described separately in instructions for use it was much easier for the laypersons to perform. Two participant of the study missed instructions in the IFU for the interpretation of a faint test tine.

SYMBOLS

Symbol	Used for	Symbol	Used for	Symbol	Used for
	Consult instructions for use		Tests per kit		Manufacturer
	In Vitro Diagnostic Medical Device		Use-by date		Do not re-use
	Store at 2℃～30℃		Catalogue number		Batch code
	Authorized Representative in the European Community		Caution		Keep dry
	Don't use the product when the package is damaged		Biological risks		n Test devices
	n Extraction tubes		n Disposable swabs		n Biohazard Waste Bag

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