BZ COVID-19 Ag Test
(Cat.No. RCOV-2050, 50Test/kit)

■ English

■ Introduction

Coronavirus is a single-stranded positive-sense RNA virus with an envelope of about 80 to 120 nm in diameter. Its genetic material is the largest of all RNA viruses and is an important pathogen of many domestic animals, pets, and human diseases. It can cause a variety of acute and chronic diseases. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. The 2019 new coronavirus, or "SARS-CoV-2 (COVID-19)", was discovered because of Wuhan Viral Pneumonia cases in 2019, and was named by the World Health Organization on January 12, 2020, confirming that it can cause colds and the Middle East Respiratory Syndrome (MERS) and more serious diseases such as acute respiratory syndrome (SARS). This kit is helpful for the auxiliary diagnosis of coronavirus infection. The test results are for clinical reference only and cannot be used as a basis for confirming or excluding cases alone.

■ Intended use

BZ COVID-19 Ag Test is a rapid and convenient immune-chromatographic assay for the qualitative detection of COVID-19 antigen (viral nucleoprotein) from nasal swab, nasopharyngeal swab obtained from patient with signs and symptoms of respiratory infection. The device is designed to aid in the rapid differential diagnosis of COVID-19 Virus infection.
This assay is not intended to be used for screening patients or as an aid for diagnosis of patients with suspected COVID-19 Ag infection, this assay is not intended for home testing (or self-testing) .The test results should be confirmed by Real-Time Reverse Transcriptase (RT)-PCR Diagnostic kit; they do not preclude COVID-19 Virus infection and should not be used as the sole basis for treatment or other management decisions. The test is intended for professional and laboratory use.

■ Test principle

BZ COVID-19 Ag Test is an antigen-capture immunochromatographic assay, detecting presence of COVID-19 viral nucleoprotein antigen in nasal swab and nasopharyngeal swab samples. This assay utilizes the chemical extraction of viral antigens followed by solid-phase immunoassay technology for the detection of extracted antigen. COVID-19 monoclonal antibodies specifically against COVID-19 antigen are conjugated with colloidal gold, deposited on the conjugate pad, and immobilized on the Test Zone of the nitrocellulose membrane. When a sample is added, the gold-antibody conjugate is rehydrated and the COVID-19 antigen, if any in the sample, will interact with the gold conjugated antibodies.

The antigen-antibody-gold complex will migrate towards the test window until the Test Zone where they will be captured by immobilized antibodies, forming a visible pink line (Test band) indicative a positive result. If COVID-19 antigen is absent in the sample, no pink line will appear in the Test Zone (T).
To serve as an internal process control, a control band was designed to indicate that the test is performed properly. By utilizing the different antigen/antibody reaction, this control line should always be seen after test is completed. Absence of a pink control line in the control region is an indication of an invalid result.

■ Kit contents

① Pouch contents: Test Cassette, Desiccant
② Reagent Solution Bottle
(includes 10ml reagent solution) (two per box)
③ Reagent Tube
④ Sterilized Nasal Swab
⑤ Test Instructions

KIT STORAGE AND STABILITY

Store the kit at 2-30℃ / 36-86°F out of direct sunlight. Kit materials are stable until the expiration date printed on the outer box. Do not freeze the kit.

WARNINGS AND PRECAUTIONS

1. Do not re-use the test kit.
2. Do not use the test kit if the pouch is damaged or the seal is broken.
3. Do not use the extraction buffer tube of another lot.
4. Do not smoke, drink or eat while handling specimen
5. Wear personal protective equipment, such as gloves and lab coats when
handling kit reagents. Wash hands thoroughly after the tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout
testing procedures.
9. Dispose of all specimens and materials used to perform the test as biohazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.

SPECIMEN COLLECTION

Freshly collected specimens should be processed as soon as possible, but no later than one hour after the specimen collection, it is essential that correct specimen collection and preparation method be followed.
Reagent, specimens and devices must be at room temperature (15-30℃) for test.
1. Insert a sterile swab into the nostril of the patient, reaching the surface of the posterior nasopharynx.
2. Swab over the surface Of the posterior nasopharynx.
3. Withdraw the sterile swab from the nasal cavity.

TEST PROCEDURES

A. Add 300ul reagent solution (approximately 9 drops) into the reagent tube
B. Add Nasal/Nasopharyngeal swab to the tube with reagent solution.
C. Vigorously rotate and twist the swab against the side of the tube at least 10 times.
D. Squeeze the sides of the tube to obtain as much liquid as possible. Dispose of swab properly.
E. Apply the cap to the tubes with specimen, and hold the tube vertically, add 5 drops (about 120 µl) of the specimen without air bubbles into the sample well.
*Note: For condensed samples, if the sample does not migrate to the membrane within 20 seconds, to apply one to two more drops of reagent solution to help the sample migrate on the membrane.
Read results at 20 minutes.

LIMITATION

1. The kit is only intended for nasal swab specimens that are collected and tested directly (i.e. swabs that have NOT been placed in transport media).
2. The kit includes a pre-diluted processing reagent in a ready to use unitized tube.
3. The kit is not INTENTED for testing liquid samples such as wash or aspirate samples or swabs in transport media as results can be compromised by over dilution.
4. Use in conjunction with the testing strategy outlined by public health authorities in your area
5. Humidity and temperature can adversely affect results.
6. The contents of this kit are to be used for the qualitative detection of COVID-19 antigen from nasal swab, nasopharyngeal swab, nasal wash and nasal aspirate specimens.
7. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.
8. Failure to follow the Test Procedure and Interpretations of Test Results may adversely affect test performance and/or invalidate the Test Result.
9. Negative test results do not rule-out possible other non-COVID-19 viral infections.
10. Positive test results do not rule out co-infections with other pathogens.
11. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.
12. Test-specific limitations, as required.

PERFORMANCE CHARACTERISTICS

■ Clinical evaluation

Total One hundred ninety eight of nasopharyngeal swab, forty eight sputum and four nasal swab samples was evaluated. Specimens collected from Korea University Guro Hospital and KCDC(2020-051). Sixty samples are COVID-19 positive and One hundred thirty eight samples are COVID-19 negative in nasopharyngeal swab. Also, Thirty eight samples are COVID-19 positive and ten samples are COVID-19 negative in sputum, two samples are COVID-19 positive and two samples are COVID-19 negative in nasal swab. In the test of clinical sensitivity and clinical specificity, BZ COVID-19 Ag Test compared to comparator method (Allplex™ 2019-nCoV Assay [RP10243X,RP10244Y]).

• Test sensitivity & specificity

The BZ COVID-19 Ag Test showed 97.0% of sensitivity and 99.3% of specificity.

1. Summary of the sensitivity and specificity of the BZ COVID-19 Ag Test compared to PCR.

		PCR		
		Positive	Negative	Total
BZ COVID-19 Ag Test	Positive	97	1	98
	Negative	3	149	152
	Total	100	150	250
Sensitivity		97.00% (95% CI : 91.48% to 99.38%)		
Specificity		99.33% (95% CI : 96.34% to 99.98%)		

ANALYTICAL PERFORMANCE

1. Sensitivity : SARS-CoV-2 virus panel was tested with BZ COVID-19 Ag Test. The LoD concentration of the virus strain was determined by 3 replicates of serial dilution test. The determined LoD concentration of the virus strain was verified with 20 additional replicates. In this study, LoD of the BZ COVID-19 Ag Test was determined as 1.6 x $10^3$$TCID_{50}$/mL.

The ten-fold serial dilution test results with three replicates for inactivated SARS-CoV-2 strain diluted sample matrix were presented in the table below:

Strain name	**Sample matrix**	**Virus Serial dilution concentration($TCID_{50}$/mL) (No. of positive /No of replicate)**			
		$1.6x10^4$	$1.6x10^3$	$1.6x10^2$	1.6x10
SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat inactivated (VR-1986HK™, Lot#70036071)	Negative nasopharyngeal swab	3/3	3/3	1/3	0/3
	Negative Nasal swab	3/3	3/3	1/3	0/3

The two-fold serial dilution test results with 20 replicates at expected LOD conc. for inactivated SARS-CoV-2 strain diluted sample matrix were presented in the table below:

Strain name	**Sample matrix**	**Virus Serial dilution concentration($TCID_{50}$/mL) (No. of positive /No of replicate)**			
		$3.2x10^3$	$1.6x10^3$	$8x10^2$	$4x10^2$
SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat inactivated (VR-1986HK™, Lot#70036071)	Negative nasopharyngeal swab	20/20	20/20	11/20	2/20
	Negative Nasal swab	20/20	20/20	13/20	3/20

2. Cross-Reactivity : The results demonstrated that BZ COVID-19 Ag Test. have no significant cross-reactivity with these specimens.

Panel information	Test concentration	#RCOV220 A01		#RCOV220 A02		#RCOV220 A03	
		Antigen: 3X LoD		Antigen: 3X LoD		Antigen: 3X LoD	
		Posi Tive	Nega tive	Posi tive	Nega tive	Posi tive	Nega tive
Adenovirus1	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Adenovirus7	1.00 × $10^{5.45}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Enterovirus 71, Tainan/4643/1998	1.60 × $10^{7.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (OC43)	2.80 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (229E)	2.80 × $10^{3.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (NL63)	1.60 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human metapneumovirus (hMPV)	2.80 × $10^{5.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Influenza A/Michigan/45/2015	1.00 × $10^{7.20}$ EID_{50}/mL	3/3	0/3	3/3	0/3	3/3	0/3
Influenza B/Wisconsin/01/2010	1.00 × $10^{7.90}$ EID_{50}/mL	3/3	0/3	3/3	0/3	3/3	0/3
MERS-Coronavirus, Irradiated Lysate	4.45 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 1	1.00 × $10^{6.86}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 2	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 3	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 4	5.00 × $10^{5.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Respiratory syncytial virus Type B	1.00 × $10^{5.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Rhinovirus	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
SARS-Coronavirus	1.00 × $10^{7.00}$ PFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Pooled human nasal wash	N/A	3/3	0/3	3/3	0/3	3/3	0/3
Bodetella pertussis	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Candida albicans	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Chlamydophila pneumoniae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Haemophilus influenzae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Legionella pneumophila	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Mycoplasma pneumoniae	1.50 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Streptococcus pneumoniae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Streptococcus pyogenes, Group A	1.00× $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3

3. Interference : All interfering substances were tested with BZ COVID-19 Ag Test. No interference reaction was observed. The results demonstrated that BZ COVID-19 Ag Test has good analytical specificity (no have cross-reactivity).

Storage condition	Storage duration	Replicate	Test results : C/T	
			Negative	SARS-CoV-2 positive (3X LoD, 4.8 x 10^3 $TCID_{50}$/mL)
			Nasopharyngeal matrix	Nasopharyngeal matrix
60 ℃	0 week (Initial test)	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	1 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	2 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	3 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	4 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	5 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	6 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
	7 week	1	4+/0	4+/2+
		2	4+/0	4+/2+
		3	4+/0	4+/2+
Continue				

4. Hook Effect : High concentration samples were tested with BZ COVID-19 Ag Test. No Hook effect was observed. All the 3 replicates were tested the range of test concentration from 1.6 x 10^5 $TCID_{50}$/mL to 1.6 x 10^3 $TCID_{50}$/mL as well as negative sample the results indicate there is no hook effect.

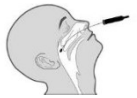
Strain name	Results	Virus serial dilution concentration ($TCID_{50}$ / mL)					
		1.6x10^5	8x10^4	4x10^4	1.6x10^4	1.6x10^3	Negative
SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat inactivated (VR-1986HK™, Lot#70036071)	Signal Intensity (C/T)	4+/5+	4+/4+	4+/3+	4+/2+	4+/1+	4+/0
		4+/5+	4+/4+	4+/3+	4+/2+	4+/1+	4+/0
		4+/5+	4+/4+	4+/3+	4+/2+	4+/1+	4+/0
	No. of positive /No of replicate	3/3	3/3	3/3	3/3	3/3	0/3

5. Stability : The accelerated stability testing during 7 weeks showed the same performance across all samples, we confirmed that the shelf life was 12 months. The acceleration test is ongoing and the stability can be extended according to subsequent test results.

Panel information	Test concentration	#RCOV220A 01		#RCOV220A 02		#RCOV220A 03	
		Antigen: 3X LoD		Antigen: 3X LoD		Antigen: 3X LoD	
		Posi tive	Nega tive	Posi Tive	Nega Tive	Posi Tive	Nega tive
Adenovirus1	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Adenovirus7	1.00 × $10^{5.45}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Enterovirus 71, Tainan/4643/1998	1.60 × $10^{7.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (OC43)	2.80 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (229E)	2.80 × $10^{3.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human coronavirus (NL63)	1.60 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Human metapneumovirus (hMPV)	2.80 × $10^{5.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Influenza A/Michigan/45/2015	1.00 × $10^{7.20}$ EID_{50}/mL	3/3	0/3	3/3	0/3	3/3	0/3
Influenza B/Wisconsin/01/2010	1.00 × $10^{7.90}$ EID_{50}/mL	3/3	0/3	3/3	0/3	3/3	0/3
MERS-Coronavirus, Irradiated Lysate	4.45 × $10^{4.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 1	1.00 × $10^{6.86}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 2	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 3	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Parainfluenza virus type 4	5.00 × $10^{5.00}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Respiratory syncytial virus Type B	1.00 × $10^{5.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
Rhinovirus	1.00 × $10^{6.20}$ $TCID_{50}$/mL	3/3	0/3	3/3	0/3	3/3	0/3
SARS-Coronavirus	1.00 × $10^{7.00}$ PFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Pooled human nasal wash	N/A	3/3	0/3	3/3	0/3	3/3	0/3
Bodetella pertussis	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Candida albicans	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Chlamydophila pneumoniae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Haemophilus influenzae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Legionella pneumophila	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Mycoplasma pneumoniae	1.50 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Streptococcus pneumoniae	1.00 × $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3
Streptococcus pyogenes, Group A	1.00× $10^{8.00}$ CFU/mL	3/3	0/3	3/3	0/3	3/3	0/3

Specimen collection

1) Insert a sterile swab into the nostril of the patient, reaching the surface of the posterior nasopharynx.



2) Swab over the surface Of the posterior nasopharynx.

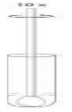
3) Withdraw the sterile swab from the nasal cavity.

Test procedure

1) Add 300ul reagent solution (approximately 9 drops) into the reagent tube

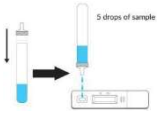
2) Add Nasal/Nasopharyngeal swab to the tube with reagent solution.

3) Vigorously rotate and twist the swab against the side of the tube at least 10 times.



4) Squeeze the sides of the tube to obtain as much liquid as possible. Dispose of swab properly.

5) Apply the cap to the tubes with specimen, and hold the tube vertically, add 5 drops (about 120 µl) of the specimen without air bubbles into the sample well.



Interpretation of test result

Re-test with a new test Cassette.

MANUFACTURER CONTACT INFORMATION

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