

EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test

This kit is designed for testing freshly collected swab samples.

INTENDED USE

EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test is intended for the simultaneous qualitative detection and differentiation of the nucleocapsid protein antigens from SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus and Adenovirus in direct nasal or nasopharyngeal swab specimens obtained from individuals, who are suspected of respiratory viral infection, within 7 days of onset of symptoms. It's an *in vitro* diagnostic test for professional use only.

The detection is based on the antibodies which were developed specifically recognizing and reacting with the nucleoprotein of virus.

Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, RSV and ADV can be similar. SARS-CoV-2, Influenza A, Influenza B, RSV and ADV viral antigens are generally detectable in upper respiratory specimens 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.

BACKGROUND

Influenza virus belongs to the family of Orthomyxoviridae, and immunologically diverse, single-stranded RNA viruses. There Influenza A and B virus is the main pathogen that severe illnesses both in human and in many animal species. Based on the current epidemiological investigation, the incubation period is 1 to 4 days. The main manifestations include acute fever, general aching and respiratory symptoms. Both Type A and B viruses can circulate simultaneously, but usually one type is dominant during a given season.

SARS-CoV-2 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel Coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

Respiratory syncytial virus was isolated from children by Chanock and Finberg in 1957. It is named human respiratory syncytial virus (HRSV) because of its special cell fusion lesions. It is reported that in 2015, there were about 331 million respiratory infections caused by HRSV worldwide, resulting in about 3.2 million hospitalized children under the age of 5, of which about 60000 died in hospital. HRSV accounts for 22% of children's acute lower respiratory tract infection in the world. The infection rate of HRSV in China is about 31/1000, accounting for 18.7% of children's acute lower respiratory tract infection. It is a virus that seriously endangers children's health.

Adenovirus is prevalent all over the world, and the epidemic law changes with region and climate. In the high-temperature tropical areas throughout the year, the prevalence of HAdV has no obvious seasonality and is scattered throughout the year. Adenovirus is generally susceptible in children, people living in a closed environment and people with low immune function.

PRINCIPLE

The **EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test** includes Influenza antigen rapid test, SARS-CoV-2/RSV antigen rapid test and ADV antigen rapid test. It is an immunochromatographic assay for the qualitative detection of 2019 Novel Coronavirus, Influenza A, Influenza B, RSV and ADV antigens.

The Influenza antigen rapid test has three letters on the surface of the strips indicating test line (Flu A), (Flu B) and control line (C). Test line and control line in the result window are not visible before

applying any samples. The control line is a reference line which indicates the test is performed properly. The control line must appear every time when the test is performed.

When the sample is added into the sample well, conjugates dried in the reagent pad are dissolved and migrate along with the sample. If Influenza A presents in the sample, a complex formed between the anti-Influenza A conjugate and the virus will be captured by the specific anti-Influenza A monoclonal antibodies coated on the Flu A region. If the sample contains Influenza B, a complex formed between the anti-Influenza B conjugate and the virus will be captured by the specific anti-Influenza B monoclonal antibodies coated on the Flu B region. Results appear at 15 minutes in the form of a red line in the Flu A region or Flu B region that develops on the membrane.

The SARS-CoV-2/RSV antigen rapid test has three letters on the surface of the strips indicating test line (RSV), (CoV) and control line (C). Test line and control line in the result window are not visible before applying any samples. The control line is a reference line which indicates the test is performed properly. The control line must appear every time when the test is performed. If SARS CoV-2 is present in the sample, the test line (CoV) would appear. The highly selective antibodies to SARS CoV-2 are used as capture and detector in the assay. These antibodies can detect SARS CoV-2 antigens directly. If RSV is present in the sample, the test line (RSV) would appear. The highly selective antibodies to RSV are used as capture and detector in the assay. These antibodies can detect RSV antigens directly.

The ADV antigen rapid test has two letters on the surface of the strips indicating test line (T) and control line (C). Test line and control line in the result window are not visible before applying any samples. The control line is a reference line which indicates the test is performed properly. The control line must appear every time when the test is performed. If ADV is present in the sample, the test line (T) would appear. The highly selective antibodies to ADV are used as capture and detector in the assay. These antibodies can detect ADV antigens directly.

CONTENTS

Test device (20), Sterilized swab (20), Extraction tube (20), Tube stand (1), Package insert (1).

STORAGE CONDITIONS

Test devices must be stored at 2~30°C. DO NOT FREEZE. Devices must be brought back to room temperature at time of testing.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Use the sterilized swab supplied in the kit for nasal/nasopharyngeal sample collection.
3. Proper specimen collection, storage and transport are critical to the performance of this test.
4. Do not use kit components beyond the expiration date.
5. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
6. Please do not touch the sample drop and the judgment part of the test board directly by hand.
7. Do not reuse the device.
8. If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
9. Assessment must be conducted exactly 15 minutes after starting the reaction. Given the nature of the measurement, the reaction and color development may slightly continue

and progress even after 15 minutes.

- The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid as long as a red line is present.
- If the line is not red at all (e.g. black), the test result is invalid and another test should be performed.
- A highly viscous specimen may affect sample migration and/or the reaction, resulting in weak coloration, delayed or no formation of the line, or a nonspecific reaction because of specimen retention.

SAMPLE COLLECTION AND PREPARATION

DOs and DON'Ts of Sample Collection

- Do use freshly collected samples of nasal swabs for optimum test performance.
- Do test sample immediately.
- Use only swabs provided with the kit.

Prepare test samples with sample extraction buffer for immediate testing after collection. If immediate testing is not possible, collected samples can be held refrigerated (2~8°C) for up to 48 hours prior to testing. Inadequate sample collection or improper sample handling may yield a false-negative result.

Nasal Swabbing

Insert nasal swab into one nostril, and the tip should be inserted up to 2.5 cm from the edge of the nostril. Gently rotate the swab 5 times or more against the nasal wall for collecting cells and mucus. Using the same swab, repeat sample collection in the other nostril.

Nasopharyngeal Swabbing

Insert sterilized swab into nostril parallel to the palate and leave in place for a few second to absorb secretions. Collect samples with nasopharyngeal (NP) swabs for optimum results.

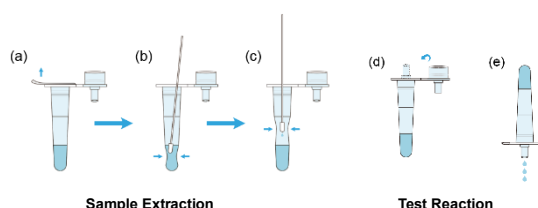


PROCEDURE

Reagents, specimens and devices must be at room temperature (15~30 °C) for testing. Please read the instruction completely before beginning to test specimens.

1. Sample Extraction

Take off the foil seal of the extraction tube and insert swab with collected sample into extraction tube containing 0.5 ml of sample extraction buffer. Squeeze the swab several times by compressing the outsidewalls of the tube end against the swab to mix well. Finally squeeze the swab to make most of the solution stays in the extraction tube and remove the swab. Use extraction solution as test sample. (step a~c)



2. Test Reaction

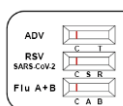
- Remove test device from sealed foil pouch prior to testing and lay flat on work bench.
- Insert the nozzle into the extraction tube with test sample (step d).
- Invert extraction tube and add 3 drops of test sample into the sample wells of SARS-CoV-2/RSV, Flu A+B and ADV by gently squeezing extraction tube (step e).
- Read results at 15 minutes and disregard after 30 minutes. A positive result may be visible at 3 minutes. However, the complete reaction time of 15 minutes is required to confirm a negative result.

INTERPRETATION OF RESULTS

Allow the samples to run according to the instruction and read the test result in the reading area.

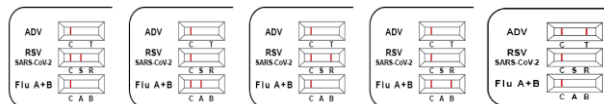
Negative Result

Only one reddish band appears on control region (C) of the device. No reddish line is visible next to the test region (T, S, R, A, B).



This indicates that there is no detectable SARS-CoV-2, RSV, ADV, Influenza A and Influenza B antigen in the sample.

Positive Result



SARS-CoV-2 (+)Flu A (+)

RSV (+)

Flu B (+)

ADV (+)

One reddish band appears in the control region (C), and one reddish band appears in the test region (T, S, R, A, B). The shade of color may vary, but it should be considered positive whenever there is even a faint band.

Influenza A positive : One reddish band in the Flu A region.

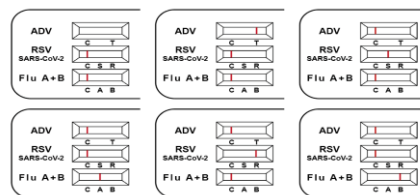
Influenza B positive : One reddish band in the Flu B region.

SARS-CoV-2 positive : One reddish band in the S region.

RSV positive : One reddish band in the R region.

ADV positive : One reddish band in the T region.

Invalid Result



No reddish band appears in the control region (C). The test is invalid even if there is a band in the test region (T, S, R, A, B). Review testing procedures and repeat the test using a new rapid test device.

QUALITY CONTROL

Internal control

Each EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test contains internal/procedural controls. The appearance of a control line at the Control "C" position validates the proper reagent function and assures that the correct test procedure was followed.

PERFORMANCE CHARACTERISTICS

Minimum detection limit

- The minimum detection limit of SARS-CoV-2 for the EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test is 140 TCID₅₀/mL.
- The minimum detection limit of Flu A for the EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was established based on a total of 8 Influenza A.

Influenza Viral Strain	Calculated LOD (TCID ₅₀ /mL)
A/New Caledonia/20/1999_H1N1	4.25x10 ³
A/California/04/2009_H1N1	2.11x10 ³
A/PR/8/34_H1N1	7.31x10 ²
A/Beau Goose/Hubei/chenhu XVI35-1/2016_H3N2	2.47x10 ²
A/Guizhou/54/89_H3N2	1.98x10 ²
A/Human/Hubei/3/2020_H3N2	1.46x10 ⁴
A/Bar-headed Goose/QH/BTY2/2015_H5N1	9.88x10 ⁴
A/Anhui/1/2013_H7N9	1.98x10 ⁵

The Flu B minimum detection limit for the EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was established based on a total of 2 Influenza B.

Influenza Viral Strain	Calculated LOD(TCID ₅₀ /mL)
B52-Victoria	39.5
B5-Yamagata	5.31x10 ²

- The minimum detection limit of RSV is as follows:

Subtype	Strain	TCID ₅₀ /mL
A	RSV (long)	6.69x10 ²
B	RSV (wild-type)	7.5x10 ²

- The minimum detection limit of ADV is as follows:

Strain	particle/mL
ADV 3	2.4x10 ³
ADV 6	4.8x10 ³
ADV 7	2.4x10 ³

High Dose Hook Effect

The signals of the test line in the test using samples with lower dilution were much higher than those with higher dilution, and no high dose at the highest dose tested HOOK effect was observed based on the trial result when the concentrations were as follows.

Virus	Subtype	Concentration (TCID ₅₀ /mL)
SARS-CoV-2	WT	1.0x10 ⁷
	Delta	3.16x10 ⁷
	Omicron	8.9x10 ⁶
Influenza A	H1N1	5.62x10 ⁷
	H3N2	2.23x10 ⁶
Influenza B	Victoria	2.51x10 ⁷
	Yamagata	7.94x10 ⁶
RSV		5.6x10 ⁷
ADV		1.92x10 ⁸ VP/mL

Precision

According to the experimental results, there was no significant difference in the detection results of different operators, or discontinuous dates, or batches, or different operation sites.

Reaction with influenza various serotype

(1) Influenza A strains:

H1N1 : A/PR/8/34, Brisbane/59/2007, California/7/2009, Fujian-Gulou/1896/2009, New Caledonia/20/1999, Solomon Island/03/2006, Aichi/2/68
H2N2 : Mallard/NY6750/78, Tokyo/3/67, Japan/305/57, H3N2 : Switzerland/9715293/2013, Victoria/3/75, Port Chalmers/1/73, Perth/16/2009, Bean Goose/Hubei/chenhu XVI35-1/2016, Guizhou/54/89,
H5N1 : Bar-headed Goose/QH/BTY2/2015,
H7N9 : Anhui/1/2013

(2) Influenza B strains :

Brisbane/72/97 Florida/2/2006
Fujian/93/97 Fukushima/220/99
Hong Kong/5/72 Johannesburg/5/99
Shanghai/04/97 B52-Victoria
B5-Yamagata

Reaction with Various Serotype of Adenovirus

The current test is able to detect the following serotype of the adenovirus: 1, 2, 3, 4, 5, 6, 7, 8, 11, 19, 37, and 55.

Clinical study

Performance of the EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was established with nasal or nasopharyngeal swabs collected from individual symptomatic patients.

All samples were tested with the EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo, in parallel with swab from each participant tested with a PCR assay. In this study, EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo demonstrated a high specificity, and a great sensitivity. For Flu A, Flu B and SARS-CoV-2 detection, samples with Ct values≤33 were counted in as positive.

Performance of Flu A Detection

		PCR (Flu A) - Nasal swab		
		+	-	Total
SARS-CoV-2 &	+	78	16	94
Flu & RSV & ADV	-	2	252	254
Antigen Combo	Total	80	268	348

Positive Percent Agreement: 97.50%

Negative Percent Agreement: 94.03%

Overall Percent Agreement: 94.83%

		PCR (Flu A) - Nasopharyngeal swab		
		+	-	Total
SARS-CoV-2 &	+	52	2	54
Flu & RSV & ADV	-	2	444	446
Antigen Combo	Total	54	446	500

Positive Percent Agreement: 96.30%

Negative Percent Agreement: 99.55%

Overall Percent Agreement: 99.20%

Performance of Flu B Detection

		PCR (Flu B) - Nasal swab		
		+	-	Total
SARS-CoV-2 &	+	77	7	84

Flu & RSV & ADV	-	2	268	270
Antigen Combo	Total	79	275	354

Positive Percent Agreement: 97.47%

Negative Percent Agreement: 97.45%

Overall Percent Agreement: 97.46%

		PCR (Flu B) - Nasopharyngeal swab		
		+	-	Total
SARS-CoV-2 &	+	39	0	39
Flu & RSV & ADV	-	1	460	461
Antigen Combo	Total	40	460	500

Positive Percent Agreement: 97.50%

Negative Percent Agreement: 100%

Overall Percent Agreement: 99.80%

Performance of SARS-CoV-2 Detection

		PCR (COVID-19) - Nasal swab		
		+	-	Total
SARS-CoV-2 & Flu & RSV & ADV	+	166	2	168
Antigen Combo	-	0	322	322
(SARS-CoV-2 Antigen)	Total	166	324	490

Positive Percent Agreement: 100%

Negative Percent Agreement: 99.38%

Overall Percent Agreement: 99.59%

		PCR (COVID-19) - Nasopharyngeal swab		
		+	-	Total
SARS-CoV-2 &	+	67	1	68
Flu & RSV & ADV	-	1	431	432
Antigen Combo	Total	68	432	500

Positive Percent Agreement: 98.53%

Negative Percent Agreement: 99.77%

Overall Percent Agreement: 99.60%

Performance of RSV Detection

		PCR (RSV) - Nasal swab		
		+	-	Total
SARS-CoV-2 &	+	110	4	114
Flu & RSV & ADV	-	3	212	215
Antigen Combo	Total	113	216	329

Positive Percent Agreement: 97.35%

Negative Percent Agreement: 98.15%

Overall Percent Agreement: 97.87%

		PCR (RSV) - Nasopharyngeal swab		
		+	-	Total
SARS-CoV-2 &	+	28	0	28
Flu & RSV & ADV	-	0	472	472
Antigen Combo	Total	28	472	500

Positive Percent Agreement: 100%

Negative Percent Agreement: 100%

Overall Percent Agreement: 100%

Performance of ADV Detection

		PCR (ADV) - Nasal swab		
		+	-	Total
SARS-CoV-2 &	+	231	11	242
Flu & RSV & ADV	-	14	855	869
Antigen Combo	Total	245	866	1111

Positive Percent Agreement: 94.29%

Negative Percent Agreement: 98.73%

Overall Percent Agreement: 97.75%

		PCR (ADV) - Nasopharyngeal swab		
		+	-	Total
SARS-CoV-2 &	+	47	0	47
Flu & RSV & ADV	-	1	452	453
Antigen Combo	Total	48	452	500

Positive Percent Agreement: 97.92%

Negative Percent Agreement: 100%

Overall Percent Agreement: 99.80%

Cross-reactivity evaluation

1. Bacteria and Yeast

The EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was found no cross reactivity with the following bacteria :

Bordetella pertussis, *Candida albicans*, *Chlamydia pneumoniae*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycobacteria tuberculosis*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*.

2. Virus

The analytical cross-reactivity was established for normal or pathogenic flora that is reasonably likely to be encountered in clinical specimens. The EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was found no cross reaction with the following virus pathogens :

Enterovirus, Human coronavirus OC43, Human coronavirus 229E, Human coronavirus NL63, Human metapneumovirus, Parainfluenza virus Type 1, Parainfluenza virus Type 2, Parainfluenza virus Type 3, Parainfluenza virus Type 4b, Rhinovirus

No cross reaction was found among SARS-CoV-2, Influenza A & B, RSV and ADV.

3. To estimate the likelihood of cross-reactivity with SARS-Coronavirus, Influenza A or B, RSV and ADV in the presence of organisms that were 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, including :

- The comparison between SARS-CoV-2 nucleocapsid protein, MERS-Coronavirus and human corona virus HKU1 revealed that cross-reactivity cannot be ruled out. Homology for HKU1 and MERS-Coronavirus is relatively low, at 36.7% across 82% of sequence and 50% across 88% of the sequence, respectively.
- The comparison between SARS-CoV-2 nucleocapsid protein and SARS-coronavirus revealed that cross-reactivity cannot be ruled out. Homology is relatively high, at 91% homology across 100% of sequences.
- No significant similarity found between *Pneumocystis jirovecii*, and SARS-CoV-2, or between *Pneumocystis jirovecii* and Influenza A or B, RSV and ADV, thus homology based cross-reactivity can be ruled out.
- No significant similarity found between SARS-Coronavirus, Influenza A or B, RSV and ADV thus homology based cross-reactivity can be ruled out.
- No significant similarity found between MERS-coronavirus, Influenza A or B, and RSV, thus homology based cross-reactivity can be ruled out.
- No significant similarity found between Human coronavirus HKU1, Influenza A or B, RSV and ADV, thus homology based cross-reactivity can be ruled out.

4. Co-infection (Competitive Interference)

For Co-infection, SARS-CoV-2 at levels near LOD was tested in the presence of high levels of Influenza A or Influenza B as well as near LOD Influenza A and Influenza B in the presence of high levels of SARS-CoV-2. No competitive interference was seen between SARS-CoV-2 and Influenza A and B in this testing at the concentration listed in the table below.

Competitive Virus	Concentration(TCID ₅₀ /mL)	Competitive Target Virus	Concentration(TCID ₅₀ /mL)	Competitive Target Percent Positivity
Influenza A	1.0 x10 ⁶	SARS-CoV-2	4.2x10 ²	100%
Influenza B	1.0 x10 ⁶	SARS-CoV-2	4.2x10 ²	100%
SARS-CoV-2	1.0 x10 ⁵	Influenza A	2.0x10 ³	100%
SARS-CoV-2	1.0 x10 ⁵	Influenza B	2.0x10 ³	100%
Influenza B	1.0 x10 ⁶	Influenza A	2.0x10 ³	100%
Influenza A	1.0 x10 ⁶	Influenza B	2.0x10 ³	100%

5. Endogenous/Exogenous Interference Substances

The EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test was found no cross reaction with the following virus pathogens :

Substance	Concentration
Whole Blood	4%
Mucin	0.3%
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
OTC Nasal Drops (Phenylephrine)	15% v/v
Afrin (Oxymetazoline)	15% v/v
OTC Nasal Spray (Cromolyn)	15% v/v
Zicam	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Sore Throat Phenol Spray	15% v/v
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Fluticasone Propionate	5% v/v
Tamiflu (Oseltamivir Phosphate)	5 mg/mL

LIMITATIONS OF THE PROCEDURE

1. This kit is a qualitative test and cannot determine the amount of antigen in the sample.
2. Users should test specimens as quickly as possible after specimen collection.
3. Positive test results do not rule out co-infections with other pathogens.
4. A false-negative test result may occur if the sample collected may contain antigen titres below the reagent's sensitivity threshold or if the sample was collected or transported improperly; therefore, a negative test result does not eliminate the possibility of the virus infection.
5. Specimen obtained early with sudden onset of symptoms will contain the highest viral titers, the amount of antigen in a sample may decrease as the duration of illness increases.
6. Failure to follow the test procedure may adversely affect test performance and/or invalidate the test result.
7. Negative results should be treated as presumptive and confirmed with a molecular assay, if necessary, for clinical management.

AVAILABILITY

Product	Cat. No.	Contents
EZER™ SARS-CoV-2 & Flu & RSV & ADV Antigen Combo Rapid Test	P211145	20 Tests

Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For in vitro diagnostic use only		Use by		Do not reuse
	Store between 2~30°C		Lot Number		Catalog #



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