

EZER™ Flu A+B Antigen Rapid Test

INTENDED USE

The *EZER™* Flu A+B Antigen Rapid Test is an *in vitro* diagnostic test for professional use only and also for the qualitative detection of influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab, throat swab and nasal aspirate samples, using the rapid immunochromatographic method. The detection is based on the monoclonal antibodies specific for the nucleoprotein of either influenza virus A or B. It is intended to aid in the rapid diagnosis of influenza A and B viral infection. Negative results should be confirmed by other methods, such as cell culture.

BACKGROUND

Influenza viruses are causative agents of highly contagious, acute, viral infections of the respiratory tract. Influenza viruses are immunologically diverse, single-stranded RNA viruses. There are three types of influenza viruses: A, B, and C. Type A viruses are the most prevalent and are associated with most serious epidemics. Type B viruses produce a disease that is generally milder than that caused by type A. Type C viruses have never been associated with a large epidemic of human disease. Both Type A and B viruses can circulate simultaneously, but usually one type is dominant during a given season.¹

Influenza illness classically presents with sudden onset of fever, chills, headache, myalgias, and a non-productive cough. Epidemics of influenza typically occur during winter months, influenza viruses can also cause pandemics, during which rates of illness and death from Influenza related complications can increase dramatically.

Patients who present with suspected influenza may benefit from treatment with an antiviral agent especially if given within the first 48 hours of onset of illness. It is important to rapidly distinguish influenza A from influenza B in order to allow physicians a choice in selective antiviral intervention. It is therefore important to not only rapidly determine whether influenza is present, but also which type of influenza virus is present.²

Diagnostic tests available for influenza include rapid immunoassay, immunofluorescence assay, polymerase chain reaction (PCR), serology, and viral culture.³⁻⁸

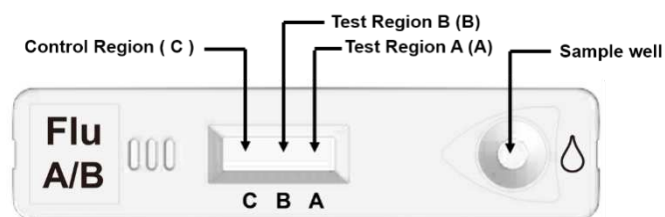
PRINCIPLE

The *EZER™* Flu A+B Antigen Rapid Test is an immuno-chromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab, throat swab and nasal aspirate samples. The test strip is composed of the following parts : Namely sample pad, reagent pad, reaction membrane, and absorbent pad. The reagent pad contains the colloidal-gold conjugated with the monoclonal antibodies against influenza virus A and

B; the reaction membrane contains the secondary polyclonal antibodies either for virus A or for B, and the polyclonal antibodies against the mouse globulin. The whole strip is fixed inside a plastic device.

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 A region (A). 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 B region (B). Results appear at 15 minutes in the form of a red line in the A region (A) or B region (B) that develops on the membrane.

To serve as a procedural control, a red line will always appear in the control region (C) indicating that proper volume of sample has been added and membrane wicking has occurred.



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STORAGE CONDITIONS

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

Warnings and Precautions

1. For *in vitro* Diagnostic Use.
2. Pathogenic microorganisms may be present in clinical specimens. All specimen and the related contaminated items need to be handled, stored and disposed following "Standard Precautions" and institutional guidelines.
3. Use the swab supplied in the kit for collection nasopharyngeal sample.
4. Proper specimen collection, storage and transport are critical to the performance of this test.
5. Do not use kit components beyond the expiration date.

6. Apply universal precaution when performing the test.
7. 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.
8. Please do not touch the sample drop and the judgment part of the test board directly by hand.
9. Do not reuse the device.
10. 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.
11. 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.
12. 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.
13. If the line is not red at all (e.g. black), the test result is invalid and another test should be performed.
14. 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

Use freshly collected samples of nasopharyngeal (NP) swabs, throat swabs and nasal aspirates for optimum test performance. Prepare test samples with sample extraction buffer for immediate testing after collection. If immediate testing is not possible, collected samples need to be held refrigerated (2-8°C) for up to 8 hours prior to testing and follow sample extraction procedures for swabs prior to refrigerating. Inadequate sample collection or improper sample handling may yield a false-negative result.



1. 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.**



2. Throat swabbing

Insert sterilized swab into throat and rotate swab gently several times to collect epidermal cells from the mucus. Avoid contaminating swab with saliva.



3. Nasal Aspiration

Collect nasal aspirate fluids using the specific aspirator as instructed.

PROCEDURE

Allow the **EZER™ Flu A+B Rapid Test** and collected samples to equilibrate to room temperature (15~30°C) prior to testing.

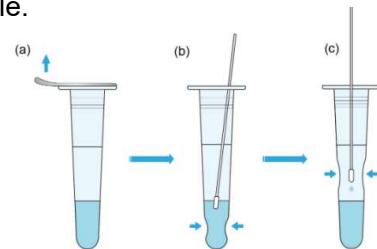
1. Sample Extraction

(1) NP or Throat swabs

Insert swab with collected sample into extraction tube containing 0.5 ml of sample extraction buffer. Squeeze the swab several times by compressing the outside walls 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) .

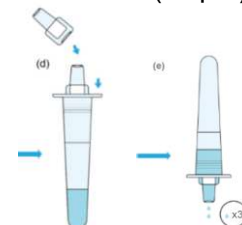
(2) Nasal aspirates

When using a nasal aspirate sampling, add 500µL of the sample to the extraction tube containing 0.5 ml of sample extraction buffer and mix well. Use extraction solution as test sample.



2. Test Reaction

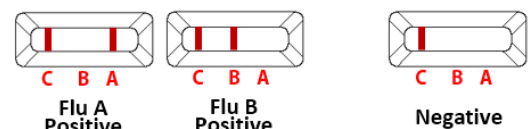
- (1) Remove test device from sealed foil pouch just prior to testing and lay flat on work bench.
- (2) Insert filtered nozzle into extraction tube containing test sample (step d).
- (3) Invert extraction tube and add 3 drops (100µl) of test sample into sample well by gently squeezing extraction tube (step e).



- (4) 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.



Influenza A Positive:

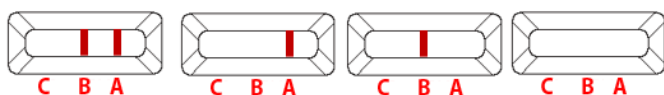
One red line appears in the control region (C), and one red line in the A region (A). The shade of color may vary, but it should be considered positive result whenever there is even a faint line.

Influenza B Positive:

One red line appears in the control region (C), and one red line in the B region (B). The shade of color may vary, but it should be considered positive result whenever there is even a faint line.

Negative:

One red line appears in the control region (C), and no line in the A region (A) or B region (B). The negative result indicates that there are no influenza A or influenza B viral particles in the samples or the number of viral particles is below the detection limit.



Invalid

Invalid:

No red line appears in the control region (C). The test is invalid even if there is a line in the A region (A) or B region (B) or both. Insufficient sample volume or incorrect procedural techniques are the most likely reasons for control line failure. Review testing procedures and repeat the test using a new rapid test device.

QUALITY CONTROL

Internal control

Each *EZER*TM Flu A+B Antigen 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

1. Detection Limit

The minimum detection limit of *EZER*TM Flu A+B Antigen Rapid Test is as follows:

Virus	TCID ₅₀ /mL
influenza A	3.0×10 ⁴
influenza B	1.5×10 ⁵

2. Cross Reactivity

No cross reaction has been confirmed of the *EZER*TM Flu A+B Antigen Rapid Test with the following pathogens: Adenovirus III type, Adenovirus VII type, ECHO virus, Coxsackie virus B₁, Coxsackie virus B₃, Coxsackie virus B₄, Coxsackie virus B₅, Coxsackie virus B₆, Herpes simplex virus I type, Respiratory syncytial virus subtype R6, Respiratory syncytial virus subtype Long, Cytomegalovirus, Rubivirus, *Chlamydia psittaci* CP₁₂, *Chlamydia psittaci* CY₁₁.

3. Clinical Study Data Summary

(1) The *EZER*TM Flu A+B Antigen Rapid Test Performance vs cell culture Techniques.

Nasal aspiration

Influenza A

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	120	2
	-	3	452
Total		123	454

Positive Percent Agreement: 97.56%
Negative Percent Agreement: 99.56%
Overall Percent Agreement: 99.13%

Influenza B

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	47	1
	-	1	528
Total		48	529

Positive Percent Agreement: 97.92%
Negative Percent Agreement: 99.81%
Overall Percent Agreement: 99.65%

Nasopharyngeal swabbing

Influenza A

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	31	1
	-	2	285
Total		33	286

Positive Percent Agreement: 93.94%
Negative Percent Agreement: 99.65%
Overall Percent Agreement: 99.06%

Influenza B

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	16	1
	-	0	302
Total		16	303

Positive Percent Agreement: 100.00%
Negative Percent Agreement: 99.67%
Overall Percent Agreement: 99.69%

Throat swabbing

Influenza A

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	22	1
	-	2	175
Total		24	176

Positive Percent Agreement: 91.67%
Negative Percent Agreement: 99.43%
Overall Percent Agreement: 98.50%

Influenza B

	cell culture		
	+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	8	1
	-	1	190
Total		9	191

Positive Percent Agreement: 88.89%
Negative Percent Agreement: 99.48%
Overall Percent Agreement: 99.00%

(2) The *EZER*TM Flu A+B Antigen Rapid Test Performance vs PCR- Fluorescent Probe Method.

Nasopharyngeal swabbing

Influenza A

		PCR- Fluorescent Probe		
		+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	102	5	107
	-	12	107	119
	Total	114	112	226

Positive Percent Agreement: 89.47%

Negative Percent Agreement: 95.54%

Overall Percent Agreement: 92.48%

Influenza B

		PCR- Fluorescent Probe		
		+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	73	3	76
	-	11	148	159
	Total	84	151	235

Positive Percent Agreement: 86.90%

Negative Percent Agreement: 98.01%

Overall Percent Agreement: 94.04%

Throat swabbing

Influenza A

		PCR- Fluorescent Probe		
		+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	71	3	74
	-	12	92	104
	Total	83	95	178

Positive Percent Agreement: 85.54%

Negative Percent Agreement: 96.84%

Overall Percent Agreement: 91.57%

Influenza B

		PCR- Fluorescent Probe		
		+	-	Total
<i>EZER</i> TM Flu A+B Antigen Rapid Test	+	63	3	66
	-	15	78	93
	Total	78	81	159

Positive Percent Agreement: 80.76%

Negative Percent Agreement: 96.30%

Overall Percent Agreement: 88.68%

LIMITATIONS OF THE PROCEDURE

1. The *EZER*TM Flu A+B Antigen Rapid Test is an acute-phase screening test for qualitative detection. Samples collected may contain antigen titres below the reagent's sensitivity threshold, so a negative test result does not exclude infection with influenza.
2. The *EZER*TM Flu A+B Antigen Rapid Test detects both viable and non-viable influenza antigens. Test performance depends on antigen loaded in the sample and may not correlate with cell culture performed on the same sample. A positive test result does not rule out the possibility that other pathogens may be present. Therefore, the test results must be compared with all other available clinical and laboratory information to make an accurate diagnosis.
3. Performance of The *EZER*TM Flu A+B Antigen Rapid Test has not been established for monitoring antiviral treatment of influenza.

REFERENCES

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Index of Symbols

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

EC REP Lotus NL B.V.
Koningin Julianaplein 10, le Verd, 2595AA,
The Hague, Netherlands.

Hangzhou Genesis
Biodetection and Biocontrol Co., Ltd.

ADD: NO.139, St.10th (East), Hangzhou Economic &
Technological Development Zone, Hangzhou,
Zhejiang Province, China, 310018
TEL: +86-571-8781-8163
FAX: +86-571-8782-4695
Web: www.genesis-ivd.com

Nasal Swab Manufacturers

 **Jiangsu HanHeng Medical Technology Co., Ltd.** **CE 0197**
16-B4, #1 North Qingyang Road, Tianning District, Changzhou,
213017 Jiangsu P.R. China

 **Luxus Lebenswelt GmbH**
Kochstr. 1, 47877, Willich, Germany

 **Jiangsu Rongye Technology Co., Ltd.** **CE 0197**
Touqiao Town, Yangzhou City 225109 Jiangsu P.R. China

 **Riomavix S.L.**
Calle de Almansa 55, 1D, Madrid 28039 Spain