EZER™ Respiratory Syncytial Virus Antigen Rapid Test

INTENDED USE

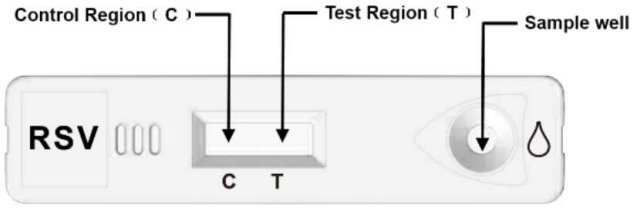
The *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test is an *in vitro* diagnostic test for the qualitative detection of Respiratory Syncytial Virus (RSV) nucleoprotein antigens in nasopharyngeal (NP) swab, throat swab and nasal aspirate samples, using the rapid immunochromatographic method. The detection is based on the monoclonal antibodies specific for the nucleoprotein of either Respiratory syncytial virus. It is intended to aid in the rapid diagnosis of RSV infection. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or for other management decisions. Negative results should be confirmed by viral culture or RSV molecular assay.

BACKGROUND

RSV is a causative agent of highly contagious, acute, viral infection of the respiratory tract in pediatric and elderly populations. Respiratory syncytial virus is a single-stranded RNA virus.[1] Nearly half of all children become infected with RSV in their first year of life. It is also the major viral cause of nosocomial illness in children already hospitalized for other reasons.[2] Worldwide, it is estimated that RSV is responsible for greater than 30 million cases of LRI in children under 5 years of age each year.[3,4] Diagnostic methods for detection of respiratory viruses include viral cell culture, direct fluorescent antibody (DFA), rapid immunoassays, and nucleic acid amplification assays such as the polymerase chain reaction (PCR).[5,6] Each has been demonstrated to have clinical utility for the detection of respiratory viruses including RSV. Rapid immunoassays available for specific viruses such as influenza A/B and RSV allow a quick diagnosis so that patients may be appropriately isolated and treated to prevent the nosocomial spread of infections to fellow patients with compromised cardiac, respiratory or immune functions.[7]

PRINCIPLE

The *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect the F protein antigen of Respiratory Syncytial Virus. The test device is composed of the following three parts, namely sample pad, reagent pad and reaction membrane. The whole strip is fixed inside a plastic device. The reagent membrane contains the colloidal gold conjugated with the monoclonal antibodies against Respiratory syncytial virus F protein; the reaction membrane contains the secondary antibodies for Respiratory syncytial virus, and the polyclonal antibodies against the mouse globulin, which are preimmobilized on the membrane. When the sample is added into the sample window, conjugates dried in the reagent pad are dissolved and migrate along with the sample. If Respiratory syncytial virus is present in the sample, a complex formed between the anti-Respiratory syncytial virus conjugate and the virus will be caught by the specific anti Respiratory syncytial virus monoclonal coated on the T region. Results appear in 15 minutes in the form of a red line that develops on the strip. Whether the sample contains the virus or not, the solution continues to migrate to encounter another reagent (an anti-mouse IgG antibody) that binds the remaining conjugates, thereby producing a red line on the region C.



Test Device

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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 or throat sample.

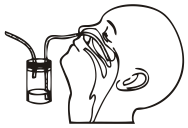
4. Proper specimen collection, storage and transport are critical to the performance of this test.
5. Do not use kit components beyond the expiration date.
6. Apply universal precaution when performing the test.
7. The test plate should be used immediately after opening the packaging. When it absorbs 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 48 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.

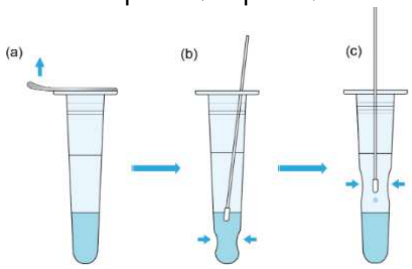
PROCEDURE

Allow the *EZER*™ Respiratory Syncytial Virus 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

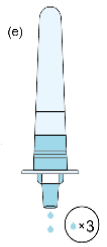
When using a nasal aspirate sampling, add 500μL of the sample to the extraction tube containing 0.5 ml of sample extraction buffer and mix well. Use extraction solution as test sample.

2. Test Reaction

(1) Remove test device from sealed foil pouch prior to testing and lay flat on work bench.

(2) Insert nozzle into the extraction tube with test sample. （step d）

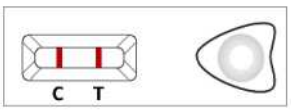
(3) Invert extraction tube and add 3 drops of test sample into the sample well by gently squeezing extraction tube. （step e）



(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.

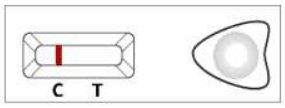
INTERPRETATION OF RESULTS

Allow the samples to react according to the procedure and read the red purple lines that appear in the reading area.



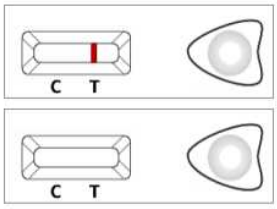
Positive

Two red lines appear. One red line appears in the control region (C), and one red line in the test region (T). This indicates that the specimen contains detectable amount of RSV antigen. The shade of color may vary，but it should be considered positive whenever there is even a faint line.



Negative

Only one red line appears in the control region of the device. No red line is visible next to the Test “T”. This indicates that there is no detectable RSV antigen in the sample.



Invalid Result

No red line appears in the control region (C). The test is invalid even if there is a line in the region (T). Review testing procedures and repeat the test using a new rapid test device.

QUALITY CONTROL

Internal control

Each *EZER*™ Respiratory Syncytial Virus rapid test device 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. Limit of Detection

The limit of detection (LOD) for the *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test is as follows：

Subtype	Strain	$TCID_{50}$/mL
A	RSV（long）	1.07×10^3
B	RSV（wild-type）	1.2×10^3

2. Reaction with Various Serotype

The current test kit is able to detect the following serotype of the Respiratory syncytial virus：Subtype A (long), Subtype B (9320, wild-type).

3. Cross Reaction

No cross reaction has been confirmed of the *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test with the following pathogens:

influenza A, influenza B, Parainfluenza 1(Grade 2), Parainfluenza 2(Grade 2), Parainfluenza 3(Grade 2), Mumps(Grade 2), Rotavirus Antigen, Measles(Grade 2), *Chlamydia pneumoniae, Chlamydia trachomatis, Escherichia coli, Pseudomonas, B. catarrhalis, Enterococcus faecalis, Citrobacter, Staphylococcus aureus, Klebsiella pneumoniae, Yersinia enterocolitica, Vibrio parahaemolyticus, Shigella flexneri, Bacteroides fragilis, Mycobacterium bovis, Mycobacterium tuberculosis, Mycobacterium avium, Salmonella enteritidis, Mycobacterium simian, Mycobacterium chelonae, Campylobacter jejuni.*

4. Specimen Matrix Interference

(1) No interference with following drug concentration up to 1 μg/mL: Dexamethasone acetate, cefradine, gentamycin sulfate, oseltamivir phosphate

(2) No interference with mucin and asthma samples.

(3) When the blood concentration in the sample is not higher than 1%, there is no interference reaction, but above this concentration, the result may be difficult to judge due to membrane staining. And even a small blood sample may cause an abnormal non-specific reaction due to the influence of plasma or blood cell components. Therefore, try to avoid getting blood when sampling.

(4) When the saliva concentration in the sample is not higher than 5%, there is no interference reaction, but above this concentration, the sensitivity may be lowered or the C line may disappear. Therefore, try to avoid saliva when sampling.

5. Clinical Study Data Summary

The *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test Performance vs Immunofluorescent test kit

Nasal aspiration

		immunofluorescence		
		+	-	Total
EZER™ Respiratory Syncytial Virus Antigen Rapid Test	+	379	11	390
	-	15	707	722
	Total	394	718	1112

Positive Percent Agreement：96.19% (95% CI：93.80%～97.85%)
Negative Percent Agreement：98.47% (95% CI：97.28%～99.23%)
Overall Percent Agreement： 97.66% (95% CI：96.59%～98.47%)

Nasopharyngeal swabbing

		immunofluorescence		
		+	-	Total
EZER™ Respiratory Syncytial Virus Antigen Rapid Test	+	110	4	114
	-	3	212	215
	Total	113	216	329

Positive Percent Agreement：97.35% (95% CI：92.44%～99.45%)
Negative Percent Agreement：98.15% (95% CI：95.33%～99.49%)
Overall Percent Agreement： 97.87% (95% CI：95.67%～99.14%)

Throat swabbing

		immunofluorescence		
		+	-	Total
EZER™ Respiratory Syncytial Virus Antigen Rapid Test	+	94	2	96
	-	2	217	219
	Total	96	219	315

Positive Percent Agreement：97.92% (95% CI：92.68%～99.75%)
Negative Percent Agreement：99.09% (95% CI：96.74%～99.89%)
Overall Percent Agreement：98.73% (95% CI：96.78%～99.65%)

LIMITATIONS OF THE PROCEDURE

• The *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test is an acute-phase screening test for qualitative detection. Sample collected may contain antigen titer below the reagent's sensitivity threshold, so a negative test result does not exclude infection with Respiratory Syncytial Virus

• The *EZER*™ Respiratory Syncytial Virus Antigen Rapid Test detects both viable and non-viable Respiratory Syncytial Virus antigen. Test performance depends on antigen load in the sample and may not correlate with cell culture performed on the same sample. A positive test does not rule out the possibility that other pathogens may be present. Therefore, the results must be compared with all other available clinical and laboratory information to make an accurate diagnosis.

AVAILABILITY

Product	Cat. No.	Contents
EZER™ Respiratory Syncytial Virus Antigen Rapid Test	P211004	20 tests

REFERENCES

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4. Nair H, Nokes DJ, Gessner BD, et al., Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet 2010;375:1545–55.
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Index of Symbols

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

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

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