

P221102**EZER™ Rotavirus Antigen Rapid Test Device****INTENDED USE**

The EZER™ Rotavirus Antigen Rapid Test Device is intended to be used to qualitatively detect rotavirus antigen in feces specimens to aid in the diagnosis of rotavirus infection in patients with diarrhea. The Test Device is intended for in vitro diagnosis only.

BACKGROUND

Rotavirus is the most common agent responsible for acute gastroenteritis, mainly in young children. Rotavirus mainly infects small intestinal epithelial cells, which causes cell damage and then causes diarrhea.

Rotavirus infections occur mainly in the summer, autumn, and winter. Rotavirus is transmitted by oral-fecal route. The clinical manifestations are acute gastroenteritis, with permeable diarrhoea disease. The course of the disease is generally 7 days, fever lasts for 3 days, vomiting for 2~3 days, diarrhea for 5 days, and severe dehydration symptoms appear.

Rotavirus (RV) is a double-stranded RNA virus belonging to the reoviridae family. There are seven species of rotavirus, designated A, B, C, D, E, F and G. A is the most common and is responsible for more than 90% of human rotavirus infections.

It is the single leading cause of diarrhea in infants and young children, and almost every child around the age of five in the world has been infected with rotavirus at least once. However, the immune system builds up after each infection and the effects of subsequent infections are lessened, so adults are less affected. Rotavirus is transmitted by oral-fecal route. Rotavirus infects enterocyte cells that link the small intestine, and produces enterotoxin, which causes gastroenteritis, severe diarrhea, and sometimes even death from dehydration.

PRINCIPLE

The EZER™ Rotavirus Antigen Rapid Test Device is a qualitative lateral flow immunochromatographic assay for the detection of rotavirus antigen in feces specimens. This product consisting of three parts: the lower part of the sample, the reagent part, and the expansion part, and has a long square-shaped carrier.

The reagent part comprises a colloidal gold-labeled anti-Rotavirus antibody A (mouse) (hereinafter referred to as colloidal gold-labeled antibody A); and the expanded part comprises an immobilized monoclonal antibody B (mouse) of Rotavirus (hereinafter referred to as Anti-Rotavirus antibody B) and murine IgG polyclonal antibody (hereinafter referred to as anti-mouse immunoglobulin antibody).

After the sample is dropped from the lower portion of the sample plate of the test plate, the colloidal gold-labeled antibody A is dissolved to form an immune complex with the Rotavirus antigen in the sample.

The immune complex moves due to the capillary phenomenon of the developing portion, and is captured by the anti-Rotavirus antibody B immobilized in the Testing portion [T], so that the Testing portion [T] forms a red-purple band due to the action of the colloidal gold.

This red-purple band of the test cassette can be visually determined to determine the presence of Rotavirus. On the other hand, regardless of whether Rotavirus is present in the sample, the remaining colloidal gold-labeled antibody A continues to move in the developing portion, and is captured by the anti-mouse immunoglobulin antibody immobilized in the Control portion [C], and red-purple band is formed by colloidal gold. This shows the normal movement of the colloidal gold marker antibody.

CONTENTS

Test Device: 20 pcs

Specimen collection tube (with sampling rod and buffer): 20 pcs

Package insert: 1 pc

WARNINGS AND PRECAUTIONS

1. For *in vitro* Diagnostic Use.
2. Do not use the device components beyond the expiration date.
3. 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.
4. Wear protective clothing such as laboratory coats, masks, disposable gloves, and eye protection when specimens are handled.
5. Ensure foil pouch containing test device is not damaged before opening for use.
6. The test device should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
7. Please do not touch the sample drop and the judgment part of the test board directly by hand.
8. Do not reuse the device.
9. Please ensure that appropriate samples are used for testing. Too much or too little sample size may lead to

deviation of results.

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. All samples and used accessories should be treated as infectious and discarded according to local regulations.

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.

SAMPLE COLLECTION AND PREPARATION

Feces specimen should be collected from symptomatic patients for Rotavirus antigen testing. Feces specimens should be collected on the first day of onset and at most not beyond the acute phase of onset (48-72 hours). If the specimens are collected long after the onset of diarrheic symptoms, the quantity of antigen may not be sufficient to obtain a positive reaction or the antigens detected may not be linked to the diarrheic episode.

The feces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives, or transport media.

Best results will be obtained if the feces specimen is tested within 6 hours after collection. Specimen collected may be stored for 48 hours at 2-8°C if not tested within 6 hours. For long-term storage, specimens should be kept below -20°C. Allow the feces specimens to equilibrate to room temperature before use.

Repeated freezing and thawing should be avoided. For non-diarrhea patients, feces specimens shall be collected at least 1-2 grams; For diarrhea patients: collect at least 1-2 ml of liquid feces.

PROCEDURE

Allow the EZER™ Rotavirus Antigen Rapid Test Device and collected samples to equilibrate to room temperature (15~30°C) prior to testing. Refer to testing procedures printed inside the Device.

Sample preparation

1. Allow the feces specimens to equilibrate to room temperature (15-30°C) before use.
2. Sample collection

- **For Solid Specimens:**

Unscrew the cap of the specimen collection tube (As shown in Figure A), then stab the specimen collection applicator into the fecal specimen to collect approximately 50 mg of feces (equivalent to 1/4 of a pea). Correct sampling method is vital for the result (As shown in Figure B).

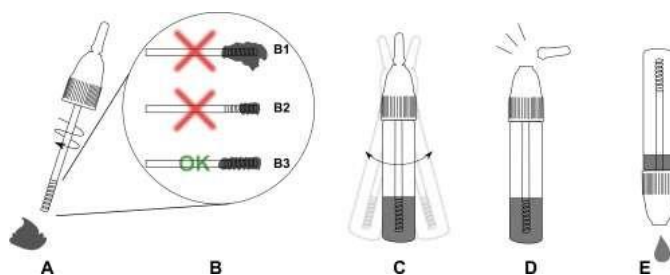
- **For Liquid Specimens:**

Hold the dropper vertically, aspirate fecal specimens, and then transfer approximately 50 µL into the specimen collection tube containing the extraction buffer.

3. Insert the screw into specimen collection tube, tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer (As shown in Figure C).

Assay procedure

1. Instructions must be read completely and allow the test cassette to equilibrate to room temperature (15-30°C) prior to testing.
2. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the feces specimen is tested within half an hour.
3. Place the test cassette on a clean, flat countertop, break off the top of the specimen collection tube (as shown in figure D), and after discarding the first two drops, add three drops (approximately 80 µl) of bubble-free sample dilution vertically to the specimen well of the test cassette. To ensure better drip, the specimen collection tube must be kept vertical (as shown in figure E).
4. Read the results at 15 minutes after dispensing the specimen.



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 Result:

A colored line appears in the control line region (C) and another colored line appears in the T line region (T).

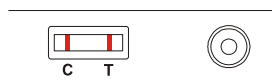
Negative Result:

One colored line appears in the control line region (C). No line appears in the test line region (T).

Invalid Result:

Control line (C) fails to appear. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test device immediately and contact your local distributor.

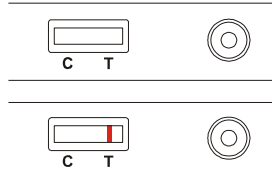
Positive



Negative



Invalid



PRECAUTIONS FOR ASSESSMENT

1. Assessment must be conducted exactly 15 minutes after starting the reaction.
2. The color tone of the line may vary depending on the color tone and specimen properties. However, the test result is valid if a red line is present.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this Device; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity

The performance of the EZER™ Rotavirus Antigen Rapid Test Device has been evaluated the clinical specimens collected from children and young adults in comparison with Rotavirus Group A, Adenovirus, Norovirus Antigen Detection Kit. The results show that the EZER™ Rotavirus Antigen Rapid Test Device has high sensitivity and specificity for rotavirus.

Result :	Rotavirus Group A, Adenovirus, Norovirus Antigen Detection Kit			
		+	-	Total
The EZER™ Rotavirus Antigen Rapid Test Device	+	213	2	215
	-	1	179	180
	Total	214	181	395

Relative Sensitivity : 99.53% (95%CI*: 97.42%~99.99%)

Relative Specificity : 98.90% (95%CI*: 96.07%~99.87%)

Accuracy : 99.24% (95%CI*: 97.80%~99.84%)

*Confidence Interval

Limit of detection

Type	Concentration
Rotavirus Antigen	0.17 µg/mL

Cross-reactivity evaluation

The EZER™ Rotavirus Antigen Rapid Test Device has been tested for no cross-reactivity with pathogenic microorganism as follow:

1. Bacteria

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Pneumobacillus*, *Branhamella catarrhalis*, *Haemophilus influenzae*, *Candida albicans*, *Neisseria meningitidis*, *Neisseria gonorrhea*, *Group B Streptococcus*, *Group C Streptococcus*, *Proteus vulgaris*, *Enterococcus faecium*, *Streptococcus faecalis*, *Bacillus mirabilis*, *Acinetobacter*, *Salmonella enteritidis*, *Escherichia coli*.

2. Viruses and Chlamydia

Norovirus, Adenovirus, Astrovirus, Coronavirus, ECHO virus, Enterovirus, Aichi virus, Coxsackie virus, Chlamydia pneumoniae, Chlamydia trachomatis.

Interfering Substances

1. Not disturbed by the color and viscosity of fecal specimens.
2. The concentration of the following substances in the sample below the level indicated in the list will not interfere with the test results.

Ascorbic acid	20mg/dL
Oxalate	100mg/dL
Hemachrome	1000mg/dL
Bilirubin	60mg/dL
Triglyceride	500mg/dL
Human serum protein	2000mg/dL

LIMITATIONS OF THE PROCEDURE

1. The EZER™ Rotavirus Antigen Rapid Test Device 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 titles 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 other assays, if necessary, for clinical management.

REFERENCES

1. Wadell, G. Laboratory Diagnosis of Infectious Diseases: Principles and Practices. New York: Springer-Verlag, Volume II, 1988: 284-300.
2. WILHELM I, ROMAN E, SANCHEZ-FAUQUIER A. Viruses causing gastroenteritis. Clin Microbiol Infect. April. 2003, vol.9:247-262
3. Cubitt, WD (1982) Rotavirus Infection: An Unexpected Hazard in Units Caring for the Elderly. Geriatric Medicine Today 1: 33-38

Index of Symbols

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



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 &
Technology Development Zone. Hangzhou,
Zhejiang Province, China, 310018

TEL : +86-571-8781-8163

FAX : +86-571-8782-4695

Web : <http://www.genesis-ivd.com>