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## Rotavirus Stool Antigen

**REF**

Catalog No. E23-387

**IVD**

*For Research Use Only*

### Intended Use

The Immunospec Rotavirus Stool Antigen Detection ELISA is an *in vitro* procedure for the qualitative determination of rotavirus antigen in feces.

### Summary

Rotavirus is one of the leading causes of gastroenteritis in children throughout the world.<sup>(2,5,7,9,11-17)</sup> Rotavirus infections are most common in infants, but repeated, asymptomatic infections are believed to occur in adults.<sup>(1,6)</sup> Rotavirus infection occurs by the fecal-oral route.<sup>(1)</sup> After an incubation period of 1-2 days, the onset of gastroenteritis is sudden. Symptoms can last from 4-5 days<sup>(6)</sup> and range from diarrhea and vomiting, to fever and occasional abdominal pain.<sup>(1,6)</sup> Loss of fluids and electrolytes can lead to severe dehydration,<sup>(1,5,6)</sup> hospitalization and even death.<sup>(1)</sup>

Rotavirus infection appears to peak during the winter season, except in countries with tropical or subtropical climates, where the virus is present year around.<sup>(17)</sup>

There have been many efforts to develop rapid and economical methods for detecting Rotavirus antigen in stool.<sup>(3,9)</sup> Simple to perform enzyme-linked immunosorbent assays (ELISA) and latex agglutination kits have been developed.<sup>(4-8)</sup> These antigen detection systems have become the test of choice in the clinical setting.<sup>(5,10,13)</sup>

### Principle of Procedure

During the first incubation, Rotavirus antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-Rotavirus antibody that "sandwiches" the antigen.

The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

### Reagents

Item	Description
Test Strips	Microwells containing anti-Rotavirus antibodies: 96 test wells in a test strip holder.
Reagent 1	One (1) bottle containing 11 ml anti-Rotavirus antibodies with blue dye and Thimerosal.
Reagent 2	One (1) bottle containing 11 ml of antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.
Positive Control	One (1) vial containing 2 ml of reactive control in buffer with Thimerosal.
Negative Control	One (1) vial containing 2 ml of buffer with Thimerosal.
Chromogen	One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal.
Stop Solution	One (1) bottle containing 11 ml of 5% phosphoric acid solution.

### Warnings/Precautions

- **Do not deviate from the specified procedures when performing this assay.** All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy.  
**Exception:** Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of

contact, flush eyes or rinse skin with copious amounts of water.

- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
- Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

#### Storage Conditions

- Reagents, strips and bottled components should be stored at 2-8 °C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C).

#### Preparation

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- ***(20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25 °C) and mixed.*** Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration. ***To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.***

#### Collection of Stool (Feces)

- Stools should be collected in clean containers.
- Unpreserved samples should be kept at 4 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 °C until used. Freezing the specimens does not adversely affect the test however, avoid repeated freeze/thaw cycles.
- All dilutions must be made with the diluted wash buffer.

#### Preparation of Sample

##### Fresh/Frozen Stools

Thaw samples if needed. Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4 ml of diluted wash buffer. Mix well and allow the heavy particulates to settle.

For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).

Note: Do not formalin fix samples prior to testing.

#### Procedure

##### Materials Provided

- Rotavirus Stool Antigen Detection Microwell ELISA Kit (see reagents on page 2)

##### Materials Required But Not Provided

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated cylinder
- Sample dilution tubes

##### Suggested Equipment

ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

#### Test Procedure

##### Notes:

- Ensure all samples and reagents are at room temperature (15-25 °C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided pre-diluted. DO NOT dilute further.

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##### Procedure:

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add **100 µl** of the negative control to well #1
3. Add **100 µl** of positive control to well #2.
4. Add **100 µl** of the stool supernatant to the appropriate test well.
5. Incubate at room temperature for **30 minutes**, then wash.\* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops** of Reagent 1 (blue solution) to each well.
7. Incubate at room temperature for **5 minutes**, then wash.\* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
8. Add **2 drops** of Reagent 2 (red solution) to each well.
9. Incubate at room temperature for **5 minutes**, then wash.\* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
10. Add **2 drops** Chromogen to each well.
11. Incubate at room temperature for **5 minutes**.

12. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
13. Read results visually or using an ELISA plate reader (see instructions below).

**\* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.**

## Results

### Interpretation of Results - Visual

**Positive:** Any sample well that is obviously more yellow than the negative control well.

**Negative:** Any sample well that is not obviously more yellow than the negative control well.

**NOTE:** The negative control, as well as some samples, may show some slight color. A sample must be obviously darker than the negative control well to be called a positive result.

### Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.

**Positive:** Absorbance reading of 0.15 OD and above indicates the sample contains Rotavirus antigen.

**Negative:** Absorbance reading less than 0.15 OD indicates the sample does not contain detectable levels of Rotavirus antigen.

### Limitation of Procedure

- Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
- DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
- A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for Rotavirus.

### Expected Results

Normal healthy individuals should be free of Rotavirus and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of Rotavirus antigen. Incidence of Rotavirus infection varies significantly between populations, season of the year, and geographic regions. No expected prevalence level can be assumed.

### Performance Characteristics

Study #1 – vs. Commercial Lateral Flow  
N=54

		Lateral Flow	
		+	-
Immunospec	+	19	1
	-	0	34

Positive Agreement: 100% (19/19)

Negative Agreement: 97.1% (34/35)

### Quality Control

The positive and negative control must be included each time the kit is run. The use of a positive and negative control allows easy validation of kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

### Troubleshooting

**Problem:** Negative control has excessive color after development.

**Reason:** Inadequate washings

**Correction:** Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out

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Revision 1

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