Rotavirus

Cat # 8306-3

Intended Use
The DAI Rotavirus Antigen Detection ELISA is an in vitro procedure for the qualitative determination of rotavirus antigen in feces. It is a double antibody (sandwich) ELISA using a polyclonal anti-rotavirus antibody to capture the antigen from the stool supernatant. A second anti-rotavirus monoclonal antibody is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color, following the addition of the chromogen and peroxide, indicates the presence of rotavirus antigens being bound by the anti-rotavirus antibodies.

Summary
Rotavirus is one of the leading causes of gastroenteritis in children throughout the world. (2,5,7,9,11-17) Rotavirus infections are most common in infants, but repeated, asymptomatic infections are believed to occur in adults. (1,6) Rotavirus infection occurs by the fecal-oral route. (1) After an incubation period of 1 - 2 days, the onset of gastroenteritis is sudden. Symptoms can last from 4 - 5 days (6) and range from diarrhea and vomiting, to fever, and occasional abdominal pain. (1,8) Loss of fluids and electrolytes can lead to severe dehydration, (1,5,6) hospitalization, and even death. (1)

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

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Strips</td>
<td>Microwells containing anti-rotavirus polyclonal antibodies - 96 test wells in a test strip holder.</td>
<td>MT PLATE</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>One (1) bottle containing 11 ml anti-rotavirus monoclonal antibodies with blue dye and Thimerosal.</td>
<td>Ab</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>One (1) bottle containing 11 ml anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.</td>
<td>CONJ</td>
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<tr>
<td>Positive Control</td>
<td>One (1) vial containing 2 ml of diluted rotavirus antigen in buffer with Thimerosal.</td>
<td>CONTROL+</td>
</tr>
<tr>
<td>Negative Control</td>
<td>One (1) vial containing 2 ml of buffer with Thimerosal.</td>
<td>CONTROL-</td>
</tr>
<tr>
<td>Chromogen</td>
<td>One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.</td>
<td>SUBS TMB</td>
</tr>
<tr>
<td>Wash Concentrate (20X)</td>
<td>Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal.</td>
<td>WASH BUF</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>One (1) bottle containing 11 ml of 1 M phosphoric acid.</td>
<td>SOLN</td>
</tr>
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Precautions
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.
Treat all reagents and samples as potentially infectious materials.

Storage Conditions
Reagents, strips and bottled components:
- Store between 2 – 8 ºC.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation
Wash/Dilution Buffer
Remove cap and add contents of one bottle of wash concentrate to 475 ml DI water. Transfer contents of diluted wash buffer into a squeeze bottle.
**Test Samples**

**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.

All dilutions must be made with the diluted wash buffer.

**Preparation of Sample**

**Fresh/Frozen Stools**
Thaw frozen stools. Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4ml 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 formalize samples prior to testing.

**Performance Of Test**

**Materials Provided**
- Rotavirus Stool Antigen Microwell ELISA Kit

**Materials Required But Not Provided**
- Pipettes
- Squeeze bottle for washing strips
- Reagent grade (DI) water
- Graduated cylinder

**Suggested Equipment**
ELISA plate reader with 450 and 620-650 nm filters

**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 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the stool supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash.*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 5 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 5 minutes, then wash.
9. Add 2 drops Chromogen to each well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.
**Interpretation of Results - Visual**

**Reactive:** Any sample well that has distinct and substantial yellow color.

**Non-reactive:** Any sample well that does not have distinct yellow color.

**NOTE:** The negative control, as well as some samples, may show some slight color.

**Interpretation of Results - ELISA Reader**

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

**Reactive:** Absorbance reading of 0.15 and above indicates the sample contains rotavirus antigen.

**Non-reactive:** Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of rotavirus antigen.

**Test Limitations**

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

**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

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<thead>
<tr>
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<th>Lateral Flow</th>
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<td>DAI</td>
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**Sensitivity –** $19/19 = 100\%$

**Specificity –** $34/35 = 97\%$

**Quality Control**

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

**Troubleshooting**

**Problem:** Negative control has substantial color development.

**Correction:** Washings were insufficient. Repeat test with more vigorous washings.
References