Rotavirus (Fecal) Antigen Detection ELISA
Cat. No. 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 AND EXPLANATION

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

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

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

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.
**WARNINGS/PRECAUTIONS**

- All incubations are at room temperature (15 to 25º C)
- Proper Temperature
- ELISA plate reader with 450 and 620-650 nm filters
- Graduated Cylinder
- Transfer Pipettes

**MATERIALS REQUIRED BUT NOT PROVIDED**

- **REAGENTS**
  - 96 Tests
  - **MICROWELL PLATE**  
    1plate  
    12x8/8x12-well strips per plate fixed on white strip holder.  
    The plate is sealed in a pouch with desiccant.  
    Each well contains anti-rotavirus polyclonal antibodies.  
    The microwell strips can be broken to be used separately.  
    Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2-8ºC.
  - **REAGENT 1**  
    1bottle  
    11ml per bottle  
    Anti-rotavirus monoclonal antibodies with blue dye and Thimerosal  
    Ready to use as supplied.  
    Once open, stable for one month at 2-8ºC.
  - **REAGENT 2 (HRP)**  
    1bottle  
    11mlL per bottle  
    Anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.  
    Ready to use as supplied.  
    Once open, stable for one month at 2-8ºC.
  - **NEGATIVE CONTROL**  
    1vial  
    1 ml per vial.  
    Dilution buffer with Thimerosal.  
    Ready to use as supplied.  
    Once open, stable for one month at 2-8ºC.
  - **POSITIVE CONTROL**  
    1vial  
    Diluted rotavirus antigen in buffer with Thimerosal.  
    Ready to use as supplied.  
    Once open, stable for one month at 2-8ºC.
  - **WASH BUFFER (20X)**  
    2 bottles  
    25ml per bottle.  
    20X concentration buffer and Thimerosal
  - **CHROMOGEN SOLUTION**  
    1bottle  
    11ml per bottle.  
    TMB (tetramethylbenzidine) and peroxide.
  - **STOP SOLUTION**  
    1bottle  
    1M phosphoric acid in water.
  - **PLASTIC SEALABLE BAG**  
    1unit  
    For enclosing the strips not in use.
  - **PACKAGE INSERTS**  
    1copy

**PROCEDURE**

**Reagent Preparation:**

1. Bring a bottle of the 20X Wash Solution to 500mL with distilled water. Mix well.

**Assay Procedure:**

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 2 drops (approximately 100 ul) of negative control to well # 1 and 2 drops of positive control to well #2.
3. Add 100ul of the stool supernatant to the appropriate test well.
4. Incubate for 30 minutes at room temperature (15-25º C), then wash.*
5. Add 2 drops of Reagent 1 (Blue soln) to each well.
6. Incubate for 5 minutes at room temperature, then wash.
7. Add 2 drops of Reagent 2 (Red soln) to each well.
8. Incubate for 5 minutes, then wash.
9. Add 2 drops of Chromogen Solution to each well.
10. Incubate at room temperature for 5 minutes, DO NOT WASH.
11. Add 2 drops of Stop Solution to each well. Mix by gently tapping the sides of the plate.
12. Read results visually or at 450/620-650 nm. Zero reader on air.

* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times.

**STORAGE CONDITIONS**

Reagents, strips and bottled components:

- Store between 2 - 8ºC.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

**SPECIMEN COLLECTION AND PREPARATION**

- **Collection of Stool (Feces)**
  1. No modification of collection techniques used for standard microscopic O&P examinations is needed. Stool samples may be used as unpreserved or frozen, or in preservation media of 10% formalin.
  2. Unpreserved samples should be kept at 2 - 4º C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at –15º to –20ºC or lower until used. Freezing does not adversely affect the test.
  3. DO NOT Formalize samples before testing.
  4. All dilutions of unpreserved stools must be made with diluted wash buffer.
  5. Wash Buffer Preparation – 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.

- **Preparation of Fresh/Frozen Stools**
  Thaw sample if needed. Add sufficient diluted wash buffer to make approximately a 1:5 dilution (1 gram or a pea size of fecal sample to 4 ml of diluted wash buffer) and mix well. Allow heavy precipitates to settle. For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).

**WARNINGS/PRECAUTIONS**

- 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.

**TRANSFER PIPETTES**

- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent grade (DI) water
- ELISA plate reader with 450 and 620-650 nm filters

**PROPER TEMPERATURE**

- All incubations are at room temperature (15 to 25º C)
RESULTS

Interpretation of Results – Visual

Reactive: Any sample well that is obviously more yellow than the negative control well.
Non-reactive: 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 well must be obviously darker than the negative control well to be called a positive result. Please refer to the enclosed visual read card for color comparisons.

Interpretation of Results – ELISA Reader
Zero reader on air. Read all wells at 450/620-650 nm.

Reactive: Absorbance reading of 0.15 OD units and above indicates the sample contains Legionella antigen.
Non-reactive: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of Legionella antigen.

QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.15 OD units. Should the value fall below this limit, the kit should not be used.

EXPECTED VALUES

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.

TROUBLESHOOTING

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings.

LIMITATION OF PROCEDURE

Excretion of Rotavirus antigen in stool may vary depending on the individual patient and the stage of the disease. Some individuals have been shown to excrete antigen for an extended period of time, so a positive ELISA reaction may reflect a recent but not active infection. Early treatment with appropriate antibiotics may also decrease antigen excretion in some individuals. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. Multiple samples over time may be indicated for those patients that are suspected of being positive for Rotavirus.

PERFORMANCE CHARACTERISTICS

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

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Sensitivity – 19/19 = 100%
Specificity – 34/35 = 97%

VALIDITY

Please do not use this kit beyond the expiration indicated on the kit box and reagent labels.

References: