

**Procedure:** Diarlex™ Rota-Adeno

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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**PRINCIPLE:**

The reagents of the Diarlex Rota-Adeno tests are dried on the cards as dry spots. These dry spot reagents consist of red latex particles affixed with antibodies against rotavirus and adenovirus antigens.

An extract is first prepared by suspension of the fecal specimen in buffer. After centrifugation or filtration of the suspension, one drop of the supernatant/filtrate is mixed with the dry spot reagents on the test card. When virus particles are present, a visible agglutination of the latex particles occurs.

**SPECIMEN:**

The fecal specimen to be tested may be formed or loose stool collected in a clean plastic container or obtained from a soiled diaper. Preservatives, heavy detergent concentrations and stool suspension media containing sera suspected of containing antibodies to rotavirus (i.e. calf or bovine sera)<sup>20</sup> or antibodies to adenovirus, have the potential to interfere with the analysis and should be avoided.

A sample volume of between 0.5 ml and 1.0 ml prior to dilution is recommended for optimal test sensitivity.

The use of rectal swabs is not recommended as the volume of stool and, consequently, the number of viral particles contained in the specimen may be inadequate for testing.

It is recommended that fecal specimens be diluted with buffer and analyzed immediately. If necessary, undiluted fecal specimens may be stored several days at 2-8°C prior to analysis.

Undiluted specimens may be frozen (below -5°C) and stored for several months. Do not freeze and thaw repeatedly or utilize a self-defrosting freezer, as these conditions may elicit erroneous results.

Meconium, as a source of specimen, has not been evaluated. It is recommended that meconium not be utilized in the Diarlex Rota-Adeno.

Since the maximum number of viral particles is present in the feces between the third and fifth day of illness, it is recommended that specimens be obtained during this interval whenever possible. Specimens collected 8 days or more after the onset of symptoms of the rotavirus disease may not contain sufficient antigen to produce a positive Diarlex Rota-Adeno test results.<sup>5,6,7</sup>

## **EQUIPMENT AND MATERIALS:**

### **Materials provided:**

Diarlex Rota-Adeno 20 + 20 tests:	
Diarlex Rota-Adeno (dry spot) Test Cards	4 packs of 5 cards
Diarlex Rota Positive Control (vial)	0.5 ml
Diarlex Adeno Positive Control (vial)	0.5 ml
Diarlex Buffer	90 ml
Mixing sticks	approx. 60 sticks
Storage bag	1 bag

### Diarlex Rota test circle:

Anti-rotavirus antibodies (rabbit) bound to red latex particles containing 0.1% sodium azide as preservative.

### Diarlex Adeno test circle:

Anti-adenovirus antibodies (rabbit) bound to red latex particles containing 0.1% sodium azide as preservative.

### Diarlex Rota-Adeno Control circle:

Preimmunized globulin (rabbit) bound to red latex particles containing 0.1% sodium azide as preservative.

### Diarlex Rota Positive Control:

Purified and diluted bovine rotavirus antigen (Nebraska Calf Diarrhea Virus, NCDV, about  $6 \times 10^7$  virions/ml), containing 0.1% sodium azide as preservative.

### Diarlex Adeno Positive Control:

Purified and diluted antigen of adenovirus serotype 3 grown in HEp2 cells (about 0.25 ul/ml), containing 0.1% sodium azide as preservative.

### Diarlex Buffer:

Tris-Buffer, 0.05 M, pH 7.2, containing 0.1% sodium azide as preservative.

**Materials required but not provided:**

Fecal Specimen Filtration Vials (Optional - Stock No. 68962) or  
Test tubes for diluting the fecal specimen  
Vortex-type mixer  
Pipettes (50 ul and 5 ml)  
Centrifuge (appr. 1000 g)

**Storage Requirements:**

Store all reagents at 2-25°C in the package provided.

Do not freeze.

NOTE: Opened test card packages containing unused test cards must be stored in the plastic storage bag provided, taking care to seal the bag tightly (do not remove the silica gel pouch from the card package!). Do not use test cards if dry spot is sticky.

**QUALITY CONTROL:**

To check the performance of the Diarlex Rota-Adeno reagents, follow the instructions below, replacing the actual diluted fecal sample suspension with one drop of the Diarlex Rota Positive Control or with the Diarlex Adeno Positive Control. These controls should be performed each day the Diarlex Rota-Adeno test is used. The Diarlex Buffer may be used as a negative control.

**PROCEDURE - STEPWISE:**

Specimen preparation

Specimen (formed or loose stools) may be prepared by using either A or B of the following procedures.

- A. Specimen treatment using the LifeSign Fecal Specimen Filtration Vial.
  - Let the sample and filtration vial reach room temperature (18-25°C).
  - Unscrew the vial cap and use the attached spoon to place two level spoonfuls (approximately 1 ml) of the fecal sample into the vial. Cap the vial and close tightly.
  - Mix the sample with the buffer by shaking the vial thoroughly. Do not use a Vortex.
  - Remove the seal at the top of the vial. Invert the vial, squeeze and discard the first five drops of diluted filtrate.
  - Proceed as described under "Use of dry latex test cards".

- B. Specimen treatment using the centrifugation technique
- Dilute the fecal specimen 1:10 with Diarlex Buffer in a test tube (e.g. one level spoonful of specimen 0.5 ml to 4.5 ml of buffer) and mix well with a Vortex-type mixer.
  - Centrifuge the suspension for approximately 10 minutes (about 1,000 g).
  - Proceed as described under "Use of dry latex test cards".

#### Use of dry latex cards

1. Open the reaction card packet by locating the corner that has been scored and pull back to remove the seal. Remove a card for testing, being careful not to touch the dried latex spots.
2. Add three drops of the supernatant, approximately 50 ul each; one onto the circle marked ROTA, one onto the circle marked ADENO and one onto the circle marked CONTROL.
3. Carefully mix the dry red spot of the circle marked ROTA with the sample drop, using the clean end of a mixing stick for each circle. Cover the entire area of the circle. Discard each stick after use.
4. Carefully mix the dry red spot of the circle marked ADENO with the sample drop, using the clean end of a mixing stick for each circle. Cover the entire area of the circle. Discard each stick after use.
5. Mix the dry red spot of the circle marked CONTROL with the sample drop carefully, using the clean end of a mixing stick for each circle. Cover the entire area of the circle. Discard each stick after use.
6. Tilt and rotate the test cards and observe the formation of agglutination within 2 minutes.

### **REPORTING RESULTS:**

#### **Interpretation of results:**

The result is positive for rotavirus when agglutination is detected in the circle labeled ROTA but not in the circle labeled CONTROL. The agglutination may be complete (red granules on white background) or partial, when granules can be detected but the background remains reddish opaque. No agglutination in the ROTA circle is a negative result.

The result is positive for adenovirus when agglutination is detected in the circle labeled ADENO but not in the circle labeled CONTROL. The agglutination may be complete (red granules on white background) or partial, when granules can be detected but the background remains reddish opaque. No agglutination in the ADENO circle is a negative result.

**Non interpretable results**

Agglutination in the CONTROL circle indicates the presence of nonspecific agglutinins in the fecal sample. Should nonspecific agglutination occur, the sample cannot be reported with this method. A new specimen may be obtained for testing or a new dilution of the original specimen may be prepared. If nonspecific agglutination persists, testing by an alternate methodology is recommended.

Consistent agglutination of all patient fecal extracts in the CONTROL may be evidence of contamination of either the buffer, filtration vial or the test cards. Such reagent is unsuitable for use and should be discarded.

**LIMITATIONS OF THE PROCEDURE:**

The Diarlex Rota-Adeno test is intended for use as an aid in the diagnosis of rotaviral or adenoviral infection. The presence of other pathogenic organisms is neither confirmed nor excluded by the results of the Diarlex Rota-Adeno test. While the causative relationship between rotavirus or adenovirus and gastroenteritis has been well established, coinfection with bacterial pathogens is possible. When bacterial coinfection is suspected, the physician may wish to consider requesting bacteriological studies to be performed in parallel with Diarlex Rota-Adeno. Therefore, it is necessary that the Diarlex Rota-Adeno test result be weighed along with the patient's clinical symptomology in determining its significance.

A negative Diarlex Rota-Adeno test result does not rule out the possibility of infection. False negative results may be a consequence of improper sampling or undetectable quantities of virus. If the sample is collected late in the course of the disease, when quantities of shed virus are low, a false negative result may occur.<sup>5,6,7,8,9,10,11,12</sup> Specimens collected 8 days or more after the onset of symptoms of the rotavirus disease may not contain sufficient antigen to produce a positive Diarlex Rota test results.<sup>5,6,7</sup>

Preservatives, heavy detergent concentrations and stool suspension media containing sera suspected of containing antibodies to rotavirus (i.e. calf or bovine sera)<sup>20</sup> or antibodies to adenovirus, have the potential to interfere with the analysis and should be avoided.

The performance characteristics were determined only on frozen samples.

Quantities of rotavirus as low as  $10^4$ /ml and of adenovirus particles  $10^8$ - $10^9$ /ml have been detected by the Diarlex Rota-Adeno test.

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**ALTERNATIVE METHOD:**

List alternative method to be used or reference laboratory should the Diarlex Rota-Adeno become unavailable.

Alternative Method:

Reference Laboratory

Name:

Address:

Address:

Phone:

Contact:

## **CRITERIA FOR REFERRAL OF SPECIMENS:**

When referring specimens for outside testing, the following procedures are recommended:

1. Verify that the testing laboratory possesses a valid CLIA certification authorizing performance of the referred test.
2. Follow laboratory guidelines for specimen shipment.
3. Report results exactly as received (no alteration or revision either of results or interpretive information provided by the testing laboratory).
4. Permit direct test report from the testing laboratory to the authorized person or entity that ordered the test.
5. Retain or be able to produce an exact duplicate of each testing laboratory's report.
6. Provide the name and address where the test was performed and indicate this information on the test report.

## **TECHNICAL ASSISTANCE:**

Technical assistance is available from the manufacturer of Diarlex Rota-Adeno LifeSign, LLC, Somerset, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. E.S.T.

1-800-526-2125