PRO-LAB DIAGNOSTICS

ROTA/ADENO COMBI-STICK

For *in vitro* diagnostic use

Product Code: PLR-5192 (25 tests)

INTENDED USE

Rota/Adeno Combi-stick is a rapid immunochromatographic test for use in the qualitative screening of human faecal samples for detection of the presence of Rotavirus or Adenovirus antigen.

SUMMARY AND EXPLANATION

Rotaviruses are one of the major causes of paediatric gastroenteritis and diarrhoea. Untreated rotavirus infection may result in severe illness with dehydration and disturbance of the body's normal electrolyte balance, especially in babies and preschool children (1). Rotavirus is the cause of up to 50% of the hospitalized cases of diarrhoeal illness in infants and young children (2). Rotavirus induced dehydration is a major cause of infant mortality in both developed and underdeveloped countries, and is a major cause of infant mortality in the latter regions (3). The highest prevalence of the disease is experienced in temperate climates during the cooler months of the year (4). In tropical climates Rotavirus infections can occur all year round (2). The age groups most susceptible to the disease are that of infants and children (4).

Adenoviruses have been implicated in a wide range of clinical diseases affectingly mainly the respiratory, ocular and the gastrointestinal systems of humans (5,6). Some Adenovirus serotypes are enteric and have emerged as a major source of paediatric gastroenteritis (7,8). The Adenoviruses are divided into six subgroups labelled A to F. Subgroup F is the most frequently involved in paediatric gastro-enteritis.

Diagnosis of Rotavirus and Adenovirus gastroenteritis is based on the identification of virus particles in the faeces. These particles, shed in large numbers during infection, may be observed by electron microscopy (EM) or detected by immunological methods, such as the immunochromatographic method used in the Rota/Adeno Combi-stick assay. The Combi-stick detects all Rotavirus and Adenovirus group viruses.

PRINCIPLE OF THE TEST

This is a ready-to-use test that is based on the use of a homogeneous immunochromatographic system with colloidal gold particles. The faecal sample must be diluted in the dilution buffer that is supplied with the test. A nitrocellulose membrane is sensitized with antibodies directed against Rotavirus and Adenovirus. The test's specificity is due to two monoclonal antibodies directed against Group A VP6 proteins of human Rotavirus and specific proteins of human Adenovirus (Hexon antigen), respectively, that are conjugated to the colloidal gold. These conjugates are insolubilized on a polyester membrane.

When the strip is dipped into the liquid phase of the faecal suspension, the solubilized mixed conjugate migrates with the sample by capillarity and the conjugate and sample material come into contact with monoclonal antibody directed against specific Adenovirus proteins. If the sample contains Adenovirus, the conjugate-Adenovirus complex remains bound to the monoclonal antibody adsorbed to the nitrocellulose and a dark red line develops. The intensity of this line depends on the sample's viral titre. The solution continues to migrate by capillarity to encounter the anti-Rotavirus polyserum that is adsorbed to the nitrocellulose. If the sample contains Rotavirus, the conjugate-Rotavirus complex will remain bound to the anti-Rotavirus polyserum and a dark red line, the intensity of which will likewise depend on the sample's viral titre, will develop. The result is visible within five to seven minutes. The solution continues to migrate to encounter a third reagent (an anti-mouse IgG antibody) that binds the surplus conjugate, thereby producing the dark red control line that confirms that the test is working properly.

REAGENTS / MATERIALS SUPPLIED

Each kit contains:

1. Rota/Adeno Combi-stick strips (25) (PLR-5193):
   Each strip is sensitized with a guinea pig anti-Rotavirus polyserum, a mouse monoclonal antibody directed against the Hexon antigens of Adenovirus groups A through F, and a goat anti-mouse IgG polyserum. The reagents are purified by affinity chromatography on protein A or G and adsorbed to the nitrocellulose.
   The anti-Rotavirus conjugate is produced with a mouse monoclonal antibody directed against the VP6 antigens of human Rotavirus group A and the anti-Adenovirus conjugate is produced with a mouse monoclonal antibody that recognizes the antigens of Adenovirus groups A, B, C, D, E, and F. The reagents are purified on protein G and coupled to colloidal gold particles.
   These strips come in a bottle of 25 with a desiccant.
2. Dilution buffer (15 ml) (PLR-5194):
   Saline solution buffered to pH 7.5 with Tris and containing EDTA, NaN_3 (<0.1%), a detergent, and charged proteins.

MATERIALS REQUIRED BUT NOT PROVIDED

3 or 5 ml test tubes
inoculating loops for taking the faecal samples.

STABILITY AND STORAGE

An unopened Combi-stick kit may be kept at between 4° and 37°C and used until the shelf-life date on the packaging. The strips and buffer solution supplied with the Combi-stick kit remain stable 18 months before being opened if they are kept in their bottles at between 4° and 37°C. The strips remain stable for 15 weeks after the bottle is opened if they are kept at between 4° and 37°C and in a dry environment. The Combi-stick kit must not be frozen.

PRECAUTIONS

1. All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
2. Rota/Adeno Combi-stick is for *in vitro* diagnostic use only.
3. Avoid touching the nitrocellulose with your fingers.
4. Wear gloves when handling the samples.
5. Dispose of gloves, swabs, test tubes, and sensitized strips in accordance with GLP.
6. Never use reagents from another kit.
7. The bottle containing the sensitized strips must be recapped as soon as the necessary number of strips for the operation has been removed, for the strips are sensitive to humidity. Make sure that the desiccant sachet is present.
8. Three green lines indicate the antibody adsorption sites. They disappear in the course of the test.
9. Avoid contamination of the dilution buffer. Discard the buffer solution if it is contaminated with bacteria or mould.
10. Do not use kit or components after expiry date shown on the product label.
11. Procedures, storage conditions, precautions and limitations specified must be adhered to in order to obtain valid test results.
12. Dilution buffer contains sodium azide which can react with lead and copper plumbing to form explosive metal azides. Azide build up may be avoided by flushing with large volumes of water following disposal of reagents.
13. The device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

SPECIMEN COLLECTION AND STORAGE

Faecal specimens should be collected in clean, dry containers, free of calf or bovine serum or detergents. Approximately 0.1 g (0.1 ml) is sufficient to perform the test. Swab samples are acceptable provided that this amount of faeces can be collected. For best results samples should be collected 3-5 days after appearance of symptoms of infection. Samples collected eight days or more after symptoms are first noted may not contain sufficient antigen or virus particles to be detected. The stool specimens must be tested as soon as possible after they are collected . If necessary, they may be stored at 2-8°C for 24 hours or -20°C for longer periods of time. Avoid repeated freezing and thawing. Make sure that the specimens are not treated with solutions containing formaldehyde or its derivatives. Samples diluted in dilution buffer should be discarded after use.

TEST PROTOCOL

Preparations:

1. If the Combi-stick kit was kept at 4°C, let all the reagents warm up to room temperature before proceeding with the test.
2. Write the patient's name or specimen number on the test tube (foresee one test tube per sample).

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3. Place the marked test tubes in a rack.

Procedure:

1. Add 0.5 ml or 15 drops of the dilution buffer solution to each tube.
2. Plunge the inoculating loop containing the stool sample into the tube. The dilution ratio must be at most 4% w/v, which equals the contents of two 10 μL loops.
3. Stir to homogenize the solution and let stand for 1-2 minutes.
4. Discard the inoculating loop and immerse the sensitized strip in the direction indicated by the red arrow. **To avoid diluting the colloidal gold conjugate in the solution, take care not to immerse the strip above the line placed under the red arrow.**
5. Let react for 5-10 minutes, with a maximum of 15 minutes. **Strongly positive samples will be detected within 1-3 minutes.**

INTERPRETATIONS OF RESULTS

The results are to be interpreted as follows:

Negative Result: A negative result is indicated by the formation of 1 dark red line at the control antibody adsorption site. (i.e. upper line)

Positive Result: Note: The intensity of the dark red lines depend on the sample's viral titre.

a) Rotavirus + Adenovirus positive: A positive result is indicated by the formation of a dark red line at each of the 3 antibody adsorption sites (i.e. 1st for Adenovirus, 2nd for Rotavirus, 3rd for Control).

b) Adenovirus positive: A positive result is indicated by the formation of dark red lines at 2 antibody adsorption sites (i.e. 1st line for Adenovirus + 3rd line for control).

c) Rotavirus positive: A positive result is indicated by the formation of dark red lines at 2 antibody adsorption sites (i.e. 2nd line for Rotavirus + 3rd line for control)

Invalid Result: An invalid result is indicated by the formation of 0 dark red lines. The absence of the control line, which is the upper line, makes the result invalid. In this case, the sample must be retested.

To store the results, let the strip dry after removing the absorbent material at its base. After drying, a very faint shadow may appear along the test line.

QUALITY CONTROL

Each test strip has a built-in control procedure. Correct device performance occurs when a dark red line appears in the control area of the strip. Good laboratory practice requires running a known positive control sample when a new lot of strips is used. If a positive result is not obtained, test results are not valid and the kit should not be used.

LIMITATIONS

1. Combi-stick kit results must be compared with all other available clinical and laboratory information.
2. A positive test does not rule out the possibility that other pathogens may be present.
3. Samples that contain detergents or preservatives are not suitable for use with this test
4. A negative result does not exclude the possibility of Rotavirus or Adenovirus infection. The quantity of virus antigen may be too small, or the sampling procedure inadequate.
5. The Combi-stick is an acute-phase screening test. Stool specimens that are collected after this phase may contain antigen titres below the reagent's sensitivity threshold.

PERFORMANCE CHARACTERISTICS

Clinical faecal specimens were tested for the presence of Rotavirus and Adenovirus in an independent hospital laboratory. The performance of Combi-stick was compared to the laboratory's reference method of electron microscopy (EM).

Rotavirus

	EM positive	EM negative
Combi-stick positive	51	0
Combi-stick negative	3	14

Relative Sensitivity (51/54) = 94.4%
Relative Specificity (14/14) = 100.0%
Agreement (65/68) = 95.6%

Note: The 3 false negative samples observed with Combi-stick were retested. Two of the 3 scored positive on retest with Combi-stick and one remained negative. These samples were low positives based on the observation that only a single virus particle was detected in each sample by EM.

Adenovirus

	EM positive	EM negative
Combi-stick positive	19	0
Combi-stick negative	3	14

Relative Sensitivity (19/22) = 86.4%
Relative Specificity (14/14) = 100.0%
Agreement (33/36) = 91.7%

Note: Two of the 3 false negative samples observed with Combi-stick were retested. Both remained negative on retest with Combi-stick. These samples were low positives based on the observation that only one virus particle was detected in each sample by EM.

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