**INTENDED USE**

The PathFlow™ Rota/Adeno Combi Cassette is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human faecal specimens to aid in the diagnosis of rotavirus or adenovirus infection.

**SUMMARY**

Acute diarrhoea disease in young children is a major cause of morbidity worldwide and is a leading cause of mortality in developing countries. 1. Rotavirus is the most common agent responsible for acute gastroenteritis, mainly in young children. In its discovery in 1973 and its association with infantile gastroenteritis represented a very important advancement in the study of gastroenteritis not caused by acute bacterial infection. Rotavirus is transmitted by oral-oral route with an incubation period of 1-3 days. Although specimen collections taken within the first 3 days of the illness are ideal for antigen detection, the rotavirus may still be found while diarrhoea continues. Rotaviral gastroenteritis may result in mortality for populations at risk such as infants, the elderly and immunocompromised patients. 2. In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported.3. With hospitalized children suffering from acute enteric disease up to 50% of the analysed specimen were positive for rotavirus.4. The viruses replicate in the cell nucleus and tend to be host species specific producing a characteristic cytopathic effect (CPE). Because rotavirus is extremely difficult to culture, it is unusual to use isolation of the virus in diagnosing an infection. Instead, a variety of techniques have been developed to detect rotavirus in faeces. Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhoea in many of these children, second only to the rotaviruses.5-8,9. These viral pathogens have been isolated throughout the world and can cause diarrhoea in children year-round. Infections are most frequently seen in children less than two years of age but have been found in patients of all ages. Further studies indicate that adenoviruses are associated with 4-15% of all hospitalized cases of viral gastroenteritis6,7,9,10. Rapid and accurate diagnosis of gastroenteritis due to adenovirus is helpful in establishing the etiology of gastroenteritis and related patient management. Other diagnostic techniques such as electron microscopy (EM) and nucleic acid hybridization are expensive and labour-intensive. With the self-limiting nature of adenovirus infection, such expensive and labor-intensive tests may not be necessary. The Rotavirus and Adenovirus Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human faecal samples, providing results in 10 minutes. The test utilizes antibody specific for rotavirus and adenovirus to selectively detect rotavirus and adenovirus from human faeces specimens.

**PRINCIPLE**

The PathFlow™ Rota/Adeno Combi Cassette is a qualitative, lateral flow immunoassay for the detection of rotavirus and adenovirus in human faecal samples.

In this test, the membrane is pre-coated with anti-rotavirus antibody on the T1 test line region of the test and anti-adenovirus antibody on the T2 test line region of the test. During testing, the specimen reacts with the particle coated with anti-rotavirus antibody and anti-adenovirus antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-rotavirus antibody and anti-adenovirus antibody on the membrane and generate a coloured line. The presence of these coloured lines in test line region indicates a positive result, while their absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

**REAGENTS**

The test contains anti-rotavirus antibody and anti-adenovirus antibody coated particles and anti-rotavirus antibody and anti-adenovirus antibody coated on the membrane.

**MATERIALS**

Provided: Test cassettes, Package Insert, Specimen collection tube with extraction buffer, Dropper.

Not Provided: Specimen collection / stool container, timer, Centrifuge and pipette to dispense 80 μL if required.

**WARNINGS AND PRECAUTIONS**

- For professional in vitro diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use test if pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being handled.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

**STORAGE AND STABILITY**

- Store as packaged at room temperature or refrigerated (2°C -30°C) prior to testing.
- The test is stable through the expiration date printed on the sealed pouch.
- The test must remain in the sealed pouch containing desiccant until use until use.
- DO NOT FREEZE.
- Do not use beyond the expiration date.

**SPECIMENS**

1. Viral detection is improved by collecting the specimens at the onset of the symptoms. It has been reported that the maximum excretion of rotavirus and adenovirus in the faeces of patients with gastroenteritis occurs 3 - 5 days after onset of symptoms. If the specimens are collected long after the onset of diarrheic symptoms, the quantity of antigen may not be sufficient to obtain a positive reaction, or the antigens detected may not be linked to the diarrheic episode.

2. The faeces specimen must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.

3. Bring the necessary reagents to room temperature before use.

**DIRECTIONS FOR USE / INSTRUCTIONS FOR USE**

Allow the test, specimen, stool collection and buffer to equilibrate to room temperature (15-30°C) prior to testing.

1. To collect faecal specimens:

   Collect sufficient quantity of faeces (1-2 ml or 1-2 g) in a clean, dry specmen collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2°C - 8°C if not tested within 6 hours. For long-term storage, specimens should be kept below -20°C.

2. To process faecal specimens:

   - For Solid Specimens:
     - Unscrew the cap of the specimen collection tube, then randomly stab the specimen collection applicator into the faecal specimen in at least 3 different sites to collect approximately 50 mg of faeces (equivalent to 1/4 of a pea). Do not scoop the faecal specimen.
     - For Liquid Specimens:
       - Hold the dropper vertically, aspirate faecal specimens, and then transfer 2 drops of the liquid specimen (approximately 50 μL) into the specimen collection tube containing the extraction buffer. Tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Leave the collection tube for reaction for 2 minutes.
       - Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
       - Hold the specimen collection tube upright and open the cap on the tip. Invert the specimen collection tube and transfer 2 full drops of the extracted specimen (approximately 80 μL) to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
       - Read the results at 10 minutes after dispensing the specimen. Do not read results after 20 minutes.
       - Note: If the specimen does not migrate (presence of particles), centrifuge the extracted specimen contained in the extraction buffer vial. Collect 80 μL of supernatant, dispense into the specimen well (S). Start the timer and continue from step 5 onwards in the above instructions for use.

**INTERPRETATION OF RESULTS**

- **Rotavirus Positive**: A coloured line appears in the control line region (C) and another coloured line appears in the T1 line region.

- **Adenovirus Positive**: A coloured line appears in the control line region (C) and another coloured line appears in the T2 line region.

- **Rotavirus and Adenovirus Positive**: A coloured line appears in the control line region (C) and two other coloured lines appear in T1 line region and T2 line region respectively.

*NOTE: The intensity of the colour in the test line region (T1/T2) will vary depending on the concentration of rotavirus or adenovirus antigens present in the specimen. Therefore, any shade of colour in the test line region (T1/T2) should be considered positive.*
**CROSS-REACTIVITY**

Cross reactivity with following organisms has been studied at 1.0 x 10^5 organisms/ml. The following organisms were found negative when tested with the Rotavirus and Adenovirus Rapid Test Cassette.

### **RELATIVE SENSITIVITY**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Proteus mirabilis</th>
<th>Neisseria gonorheaea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
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<tr>
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<td></td>
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<tr>
<td>Enterococcus faecalis</td>
<td></td>
<td></td>
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<tr>
<td>Group C Streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td></td>
<td></td>
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<tr>
<td>Branhamella catarrhalis</td>
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### **RELATIVE ACCURACY**

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**TABLE OF SYMBOLS**

<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>LOT</td>
<td>Batch Code/Lot Number</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue Reference</td>
</tr>
<tr>
<td>CE</td>
<td>CE Mark</td>
</tr>
<tr>
<td>Caution</td>
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</table>

This document applies to the following product codes: M595CE / M595a / M595b