PathFlow™ Rota/Adeno Combi (EN)

MICROGEN BIOPRODUCTS

Instructions for Use

INTENDED USE

The PathFlow™ Rota/Adeno Combi Cassette is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human faeces 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[2]. 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-faecal route with an incubation period of 1-3 days. Although specimen collections taken within the second and fifth day 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[3]. In temperate climates, rotavirus infections occur mainly in the winter months. Endemics as well as epidemics affecting some thousand people have been reported[4]. With hospitalized children suffering from acute enteric disease up to 50% of the analysed specimen were positive for rotavirus[5]. 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[6,7,8,9].

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 gastroenteritis[5,6,7,8,9].

Rapid and accurate diagnosis of gastroenteritis due to adenovirus is helpful in establishing the ethology 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 labour-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 faeces specimen, 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 faeces specimen.

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 tested.
- 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).
- 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 specimen 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:

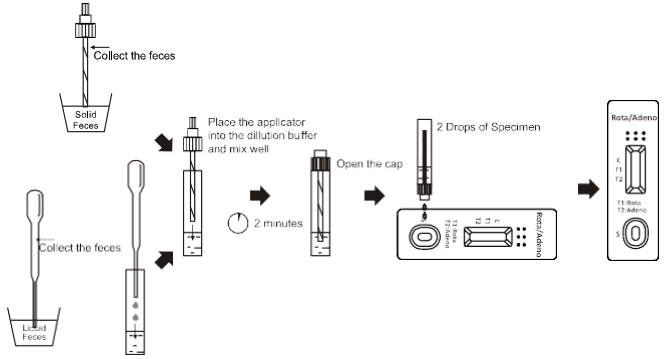
   • For Solid Specimens:

   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.
3. 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.
4. 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.
5. 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.

	**Negative**: One pink line in the Control (C) region shows that the test has been carried out correctly. If there is no line in the Test region the sample is negative for both adenovirus and rotavirus.
	**INVALID**: Control line (C) fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

An internal procedural control is included in the test. A coloured line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The Rotavirus and Adenovirus Rapid Test Cassette is for in vitro diagnostic use only. The test should be used for the detection of human rotavirus and adenovirus in faeces specimens only. Neither the quantitative value nor the rate of increase in human rotavirus and adenovirus concentration can be determined by this qualitative test.
2. The Rotavirus and Adenovirus Rapid Test Cassette will only indicate the presence of rotavirus and adenovirus in the specimen and should not be used as the sole criteria for the conforming rotavirus and adenovirus to be etiological agent for diarrhoea.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of rotavirus or adenovirus infection with low concentration of virus particles.

CLINICAL SENSITIVITY, SPECIFICITY AND ACCURACY

The performance of the Rotavirus and Adenovirus Rapid Test Cassette has been evaluated the clinical specimens collected from children and young adults in comparison with latex agglutination method. The results show that the Rotavirus and Adenovirus Rapid Test Cassette has high sensitivity and specificity for rotavirus and adenovirus.

Method	Results	Latex Agglutination		Total Results
		Positive	Negative	
Rotavirus rapid test	Positive	251	7	**258**
	Negative	7	236	**243**
Total Results		**258**	**243**	**501**

Relative Sensitivity: 97.3% (95%CI:*94.5%-98.9%)
Relative Specificity: 97.1% (95%CI:*94.2%-98.8%)
Relative Accuracy: 97.2% (95%CI:*95.4%-98.5%) *Confidence Intervals

Method	Results	Latex Agglutination		Total Results
		Positive	Negative	
Adenovirus rapid test	Positive	118	6	**124**
	Negative	6	251	**257**
Total Results		**124**	**257**	**381**

Relative Sensitivity: 95.2% (95%CI:*89.8%-98.2%)
Relative Specificity: 97.7% (95%CI:*95.0%-99.1%)
Relative Accuracy: 96.8% (95%CI:*94.6%-98.4%) *Confidence Intervals

CROSS-REACTIVITY

Cross reactivity with following organisms has been studied at 1.0 x 10^9 organisms/ml. The following organisms were found negative when tested with the Rotavirus and Adenovirus Rapid Test Cassette.

Staphylococcus aureus	Proteus mirabilis	Neisseria gonorrhea
Pseudomonas aeruginosa	Acinetobacter spp	Group B Streptococcus
Enterococcus faecalis	Salmonella choleraesius	Proteus vulgaris
Group C Streptococcus	Gardnerella vaginalis	Enterococcus faecium
Klebsiella pneumoniae	Acinetobacter calcoaceticus	Hemophilus influenzae
Branhamella catarrhalis	E.coli	Neisseria meningitidis
Candida albicans	Chlamydia trachomatis	

INTERFERING SUBSTANCES

The following potentially Interfering Substances were added to Adenovirus negative and positive specimens.

Ascorbic acid:20mg/dl	Oxalic acid: 60mg/dl	Bilirubin: 100mg/dl
Uric acid: 60mg/dl	Aspirin: 20mg/dl	Urea: 2000mg/dl
Glucose: 2000mg/dl	Caffeine:40mg/dl	Albumin: 2000mg/dl

EXPECT VALUE

The Rotavirus and Adenovirus Rapid Test Cassette has been compared with latex agglutination method, demonstrating an overall accuracy of ⩾97.0%.

PRECISION INTRA-ASSAY

Within-run precision has been determined by using 10 replicates of seven specimens: a negative, a rotavirus low positive, an adenovirus low positive, a rotavirus medium positive, an adenovirus medium positive, a rotavirus high positive and an adenovirus high positive.

The specimens were correctly identified >99% of the time.

INTER-ASSAY

Between-run precision has been determined by 10 independent assays on the same seven specimens: a negative, a rotavirus low positive, an adenovirus low positive, a rotavirus medium positive, an adenovirus medium positive, a rotavirus high positive and an adenovirus high positive.

The specimens were correctly identified >99% of the time.

BIBLIOGRAPHY

1. Wadell, G. Laboratory Diagnosis of Infectious Diseases: Principles and Practices. New York: Springer-Verlag, Volume II, 1988: 284-300.
2. WILHELMI I, ROMAN E, SANCHEZ-FAUQUIER A. Viruses causing gastroenteritis. Clin Microbiol Infect. April. 2003, vol.9:247-262
3. Cubitt, WD (1982) Rotavirus Infection: An Unexpected Hazard in Units Caring for the Elderly. Geriatric Medicine Today 1: 33-38
4. Hung, T et al (1984) Waterborne outbreak of Rotavirus Diarrhoea in Adults in China caused by a Novel Rotavirus. Lancet, May 26;1(8387): 1139-1142
5. Cukor, G; Perron, DM; Hudson, R and Blacklow, NR (1984) Detection of Rotavirus in Human Stools by Using Monoclonal Antibody. J. Clin. Microl. 19: 888-892
6. Wood, D. J. and A. S. Bailey. "Detection of Adenovirus Types 40 and 41 in Stool Specimens by Immune Electron Microscopy." Journal of Medical Virology, 1987; 21: 191-199.
7. Nishio, Osamu, M. Ooseto, K. Takagi, Y. Yamasita, Y. Ishihara, and S. Isomura. "Enzyme-Linked Immunosorbent Assay Employing Monoclonal Antibodies for Direct Identification of Enteric Adenoviruses (Ad40, 41) in Faeces." Microbiol. Immunol. 1990; 34(10): 871-877.
8. Wood, D. J., K. Bijlsma, J. C. de Jong, and C. Tonkin. "Evaluation of a Commercial Monoclonal Antibody-Based Enzyme Immunoassay for Detection of Adenovirus Types 40 and 41 in Stool Specimens." Journal of Clinical Microbiology, June 1989; 27(6): 1155-1158.
9. Thomas, Eva. E., D. Roscoe, L. Book, B. Bone, L. Browne, and V. Mah. "The Utility of Latex Agglutination Assays in the Diagnosis of Pediatric Viral Gastroenteritis." Am. J. Clin. Pathol. 1994; 101:742-746.

TABLE OF SYMBOLS

SYMBOL	DEFINITION
LOT	Batch Code/Lot Number
IVD	In Vitro Diagnostic Medical Device
REF	Catalogue Reference
	Upper and Lower Temperature Limit
	Expiry Date
	Manufacturer and Address Details
	Date of Manufacture
	Please consult Instructions for Use
	Sufficient for
	Do not use if package is damaged
CE	CE Mark
	Caution

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

Microgen Bioproducts Limited, Unit 1, Watchmoor Point, Camberley, Surrey, GU15 3AD, United Kingdom.
www. http://microgenbioproducts.com/
Email: enquiries.microgen@novacyt.com
Tel: +44 (0) 1276 600081
Fax: +44 (0) 1276 600151
Part of the Novacyt Group

CE