Ref: SW40001

BIOSYNEX® Adenovirus/Rotavirus BSS

Rapid test for the qualitative detection of Rotavirus and Adenovirus in human feces

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1. INTENDED USE

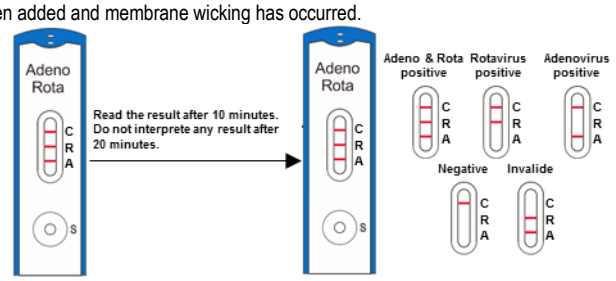
BIOSYNEX® Adenovirus/Rotavirus BSS is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human feces samples. This test is intended to be used as an aid in the diagnosis of rotavirus or adenovirus infections.

2. INTRODUCTION

Acute diarrhea 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-fecal route with an incubation period of 1-3 days. Although sample collections taken within the second and fifth day of the illness are ideal for antigen detection, the rotavirus may still be found while diarrhea 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 analyzed 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 to diagnose an infection. Instead, a variety of techniques have been developed to detect rotavirus in feces. Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhea in many of these children after rotaviruses.[6,7,8,9] These viral pathogens have been isolated worldwide, and can cause diarrhea 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 caused by 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 labor-intensive. With the self-limiting nature of adenovirus infection, such expensive and labor-intensive tests may not be necessary.
BIOSYNEX® Adenovirus/Rotavirus BSS is a rapid chromatographic immunoassay for the qualitative detection of rotavirus and adenovirus in human feces samples, providing results in 10 minutes. The test utilizes antibodies specific for rotavirus and adenovirus to selectively detect rotavirus and adenovirus from human feces samples.

3. PRINCIPLE

The BIOSYNEX® Adenovirus/Rotavirus BSS test cassette is a qualitative, lateral flow immunoassay for the detection of rotavirus and adenovirus in human feces samples.
In this test, the membrane is pre-coated with anti-rotavirus antibodies on the (R) test line region of the test and anti-adenovirus antibodies on the (A) test line region of the test. During testing, the positive sample reacts with the particle-coated anti-rotavirus antibodies and anti-adenovirus antibodies in the sample well (S). The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-rotavirus antibodies and anti-adenovirus antibodies on the membrane and generate a colored line.
The presence of these colored lines in the test line regions indicates a positive result, while their absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region (C) indicating that proper volume of sample has been added and membrane wicking has occurred.



4. REAGENTS

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

5. MATERIALS PROVIDED

- Test cassettes in individually sealed pouches
- Sample collection tubes with extraction buffer
- Droppers
- Package insert

6. MATERIALS REQUIRED BUT NOT PROVIDED

- Sample collection containers
- Centrifuge and pipette to dispense 80 µL, if required
- Timer

7. STORAGE AND STABILITY

Store as packaged in the sealed pouches either at room temperature or refrigerated (2°C - 30°C). The test is stable through the expiration date printed on the sealed pouch. However, the reagents can be stored at 55°C for 42 days or at 45°C for 84 days without altering the shelf life and quality of the product. DO NOT FREEZE. Do not use beyond the expiry date.

8. PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiry date.
- The test cassette should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Do not use test if pouch is damaged.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow 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.

9. SAMPLE COLLECTION AND PREPARATION

- Viral detection is improved by collecting the samples at the onset of the symptoms. It has been reported that the maximum excretion of rotavirus and adenovirus in the feces 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.
- The feces sample must be collected in a clean, dry, waterproof container containing no detergents, preservatives.

Raw stools: stool samples can be stored at room temperature for 6 hours or at 2°C to 8°C for 3 days. For prolonged storage, samples may be stored at -20 ° C for 6 months.
Sample diluted in the extraction buffer: once the stool sample has been diluted in the extraction buffer bottle, this vial can be kept at room temperature for 6 hours or at 2°C to 8°C for 3 days. For longer storage, the vial may be stored at -20 ° C for 6 months.
Note: Samples can also be stored in Transwab® transport medium (Fecal Transwab® - REF MW168S) for 1 hour at room temperature, for 7 days at 2-8°C or for 7 days at -20°C.

10. PROCEDURE

Bring the test cassette, specimen, and extraction buffer to reach room temperature (15°C - 30°C) prior to testing.

Fecal samples collection and pre-treatment

1) Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry sample collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours following sample collection.
2) For Solid Specimens:
   Unscrew the green cap of the sample collection tube to access the applicator. Randomly stab the applicator into the fecal sample in at least 3 different sites to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
   For Liquid Specimens:
   Hold the dropper vertically, aspirate fecal sample, then transfer 2 drops of the liquid sample (approximately 50 µL) into the sample collection tube containing the extraction buffer.

Recap the sample collection tube, then shake vigorously to mix the sample and the extraction buffer. If the diluted sample is to be tested immediately, let the tube to stand for 2 minutes (at least 1 minute 30 seconds) prior to testing. For delayed use, the diluted sample may be stored at room temperature for up to 6 hours.

3) Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it within the hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
4) Unscrew the colorless cap of the sample collection tube to access the dropper. Hold the sample collection tube vertically, invert the tube and transfer 2 full drops of extracted sample (approximately 80 µL) into the sample 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**. Do not read results after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the specimen contained in the extraction buffer tube at 3,000-5,000 rpm for 5 to 10 minutes. Collect 80 µL of supernatant, dispense into the specimen well (S). Start the timer and continue from step 5 onwards of these instructions for use.

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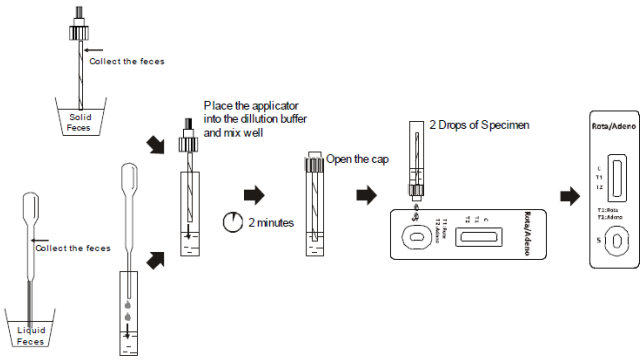


11. RESULTS INTERPRETATION

Positive result for Adenovirus, Rotavirus or both viruses :

Rotavirus Positive: A colored line appears in the control line region (C) and another colored line appears in the (R) line region.

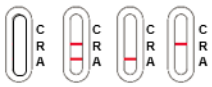
Adenovirus Positive: A colored line appears in the control line region (C) and another colored line appears in the (A) line region.

Rotavirus and Adenovirus positive: A colored line appears in the control line region (C) and two other colored lines appear in the (R) and (A) line regions.

Note: The intensity of the color in the test line regions (R/A) may vary depending on the concentration of rotavirus or adenovirus antigens present in the sample. Therefore, any shade of color in the test line regions (R/A) should be considered positive.

Negative result

Negative: One colored line appears in the control line region (C). No line appears in the test line regions (R/A).



Invalid: Control line (C) fails to appear. Insufficient sample 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.

12. QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal positive procedural control. It confirms sufficient sample volume, adequate membrane wicking and correct procedural technique.
Control standards are not supplied with this kit; however. However, Good Laboratory Practice recommends to test controls in order to confirm the test procedure and check the performance of the test.

13. LIMITATIONS

- The BIOSYNEX® Adenovirus/Rotavirus BSS test is for *in vitro* diagnostic use only. The test should be used for the detection of human rotavirus and adenovirus in feces samples only. Neither the quantitative value nor the rate of increase in human rotavirus and adenovirus concentration can be determined by this qualitative test.
- The BIOSYNEX® Adenovirus/Rotavirus BSS test will only indicate the presence of rotavirus and adenovirus in the sample and should not be used as the sole criteria for the conforming rotavirus and adenovirus to be etiological agent for diarrhea.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- 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.
- Some non specific reactions were documented in newborns and could rarely be seen in adults : in any case, the presence of both adenovirus and rotavirus lines should be interpreted carefully.

14. EXPECTED VALUES

The BIOSYNEX® Adenovirus/Rotavirus BSS test has been compared with latex agglutination method, demonstrating an overall accuracy of ≥97.0%.

15. PERFORMANCE CHARACTERISTICS

➢ **Clinical Sensitivity, Specificity and Accuracy**

The performance of the BIOSYNEX® Adenovirus/Rotavirus BSS test has been evaluated with clinical samples collected from children and young adults in comparison with latex agglutination method. The results show that the BIOSYNEX® Adenovirus/Rotavirus BSS test has high sensitivity and specificity for rotavirus and adenovirus.

		Latex agglutination		Total
		+	-	
Rotavirus rapid test	+	251	7	258
	-	7	236	243
	Total	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*

		Latex agglutination		Total
		+	-	
Adenovirus rapid test	+	118	6	124
	-	6	251	257
	Total	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*

➢ **Precision**

- Intra-Assay:

Within-run precision has been determined by using 10 replicates of seven samples: 1 negative, 1 rotavirus low positive, 1 adenovirus low positive, 1 rotavirus medium positive, 1 adenovirus medium positive, 1 rotavirus high positive and 1 adenovirus high positive. The samples were correctly identified >99% of the time.

- Inter- Assay:

Between-run precision has been determined by 10 independent assays on the same seven specimens: 1 negative, 1 rotavirus low positive, 1 adenovirus low positive, 1 rotavirus medium positive, 1 adenovirus medium positive, 1 rotavirus high positive and 1 adenovirus high positive. The specimens were correctly identified >99% of the time.

➢ **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 BIOSYNEX® Adenovirus/Rotavirus BSS test.

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*	*Group A Streptococcus*
Helicobacter pylori		

➢ **Interfering substances**

The following potentially Interfering Substances were added to negative sample, Rotavirus middle positive sample and Adenovirus middle positive sample at the concentrations listed:

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

No substances showed any interference with the BIOSYNEX® Adenovirus/Rotavirus BSS test.

➢ **Hook effect**

11 Rotavirus positive specimens (from 1.0 x 10^6 organisms/mL to 2.5 x 10^{10} organisms/mL) and 11 Adenovirus positive specimens (from 1.0 x 10^6 organisms/mL to 1.92 x 10^{10} organisms/mL) were tested in replicates of three with the BIOSYNEX® Adenovirus/Rotavirus BSS test. No dose hook effect has been observed.

➢ **Detection limit**

A detection limitation study of the BIOSYNEX® Adenovirus/Rotavirus BSS test has been performed. This study shows that the detection limit of the BIOSYNEX® Adenovirus/Rotavirus BSS test of 1.0 x 10^5 $TCID_{50}$/mL for G1, G2, G3, G4 serotypes in group A rotaviruses and of 1.0 x 10^5 $TCID_{50}$/mL for types 40 and 41 adenoviruses.

16. BIBLIOGRAPHIE

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SYMBOLES

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
2°C 30°C	Store between 2°C - 30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
DIL	Diluent / Extraction buffer		Use by

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