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MATERIAL SAFETY DATA SHEET

Adenovirus

IDENTIFICATION OF THE PREPARATION AND COMPANY NAME.

Supplier: Diagnostic Automation Inc.
21250 Califa Street, Suite 102 and 116,
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Emergency Telephone number: Please contact the local hospitals.

Product Name: Adenovirus Antigen Detection ELISA
Catalog No: 8300-3

Intended Use

The Diagnostic Automation Inc. Adenovirus Antigen Detection ELISA is an *in vitro* procedure for the qualitative determination of adenovirus antigen in feces.

Summary

Acute diarrheal disease in young children is a major cause of morbidity world wide and is a leading cause of mortality in developing countries ⁽⁸⁾. Research has shown that enteric adenoviruses, primarily Ad40 and Ad41, are a leading cause of diarrhea in many of these children, second only to the rotaviruses. ^(1,3,5-8) These

viral pathogens have been isolated throughout the world, and can cause diarrhea in children year round.⁽¹⁻⁴⁾ Infections are most frequently seen in children under two years of age,⁽¹⁻³⁾ but have been found in patients of all ages.⁽²⁾ Further studies indicate that adenoviruses are associated with 4 - 15% of all hospitalized cases of viral gastroenteritis.⁽¹⁻⁸⁾

Adenoviruses have an incubation period of 8 - 10 days,⁽¹⁻³⁾ followed by viral shedding for an approximate period of 7 - 14 days.⁽¹⁻³⁾ The main symptoms are diarrhea⁽¹⁻⁴⁾ and vomiting,⁽³⁾ however a fever is also seen in 40 - 90% of the cases.⁽³⁾ The diarrhea resulting from enteric adenoviruses is longer in duration than that caused by the rotaviruses, usually lasting 7 - 8 days.⁽³⁾ This is a leading reason why patients seek medical attention for this condition.⁽¹⁾ Viral transmission is believed to be by the fecal- oral route.⁽³⁾

Viral gastroenteritis is usually self-limiting, but accurate diagnosis can eliminate the need for more expensive and invasive diagnostic tests. Many laboratories use electron microscopy (EM) to detect viruses associated with gastroenteritis^(5, 7, 8). This technique is expensive, labor-intensive, and not readily available⁽⁸⁾. Other techniques include direct genome proliferating and nucleic acid hybridization, neither of which is rapid or specific⁽⁶⁾. Alternatively, ELISA tests using Ad-specific antibodies have been shown to be a sensitive⁽⁹⁾, specific, and rapid diagnostic method for the determination of enteric adenoviruses⁽⁶⁾.

Principle of Procedure

During the first incubation, adenovirus antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-adenovirus antibody that “sandwiches” the antigen. The third incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

Item	Description	Symbol
Test Strips	Microwells containing anti-adenovirus polyclonal antibodies- 96 test wells in a test strip holder.	MT PLATE
Reagent 1	One (1) bottle containing 11 ml anti-adenovirus monoclonal antibodies with blue dye and Thimerosal.	Ab

Reagent 2	One (1) bottle containing 11 ml anti-mouse antibodies conjugated to horseradish peroxidase with red dye and Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted adenovirus antigen in buffer with Thimerosal.	CONTROL +
Negative Control	One (1) vial containing 2 ml of buffer with Thimerosal.	CONTROL -
Chromogen	One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and Thimerosal.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 1 Mphosphoric acid.	SOLN

Precautions

Do not use solutions if they precipitate or become cloudy.

Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.

Do not add azides to the samples or any of the reagents.

Controls and some reagents contain Thimerosal as a preservative. Treat all reagents and samples as potentially infectious materials.

Storage Conditions

Reagents, strips and bottled components:

Store between 2-8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation

Remove cap and add contents of one bottle of wash concentrate to 475 ml DI water. Transfer contents of diluted wash buffer into a squeeze bottle.

Test Samples

Collection of Stool (Feces)

Stools should be collected in clean containers.
Samples should be kept at 4 °C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 °C until used.
Freezing the specimens does not adversely affect the test.
All dilutions must be made with the diluted wash buffer.

Preparation of Sample

Fresh/Frozen Stool

Thaw frozen stools. Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4ml of diluted wash buffer. Mix well and allow the heavy particulates to settle.
For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution). Note: Do not formalize samples prior to testing.

Performance Of Test

Materials Provided

Adenovirus Stool Antigen Microwell ELISA Kit

Materials Required But Not Provided

Pipettes
Squeeze bottle for washing strips
Reagent grade (DI) water
Graduated cylinder

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters (optional if results are read visually).

Procedure

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the stool supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash. *
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 5 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 5 minutes, then wash.
9. Add 2 drops Chromogen to each well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.

12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

Interpretation of Results - Visual

Reactive: Any sample well that has distinct and substantial yellow color.

Non-reactive: Any sample well that does not have distinct yellow color.

NOTE: The negative control, as well as some samples, may show some slight color.

Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

Reactive: Absorbance reading of 0.15 and above indicates the sample contains adenovirus antigen.

Non-reactive: Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of adenovirus antigen.

Test Limitations

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

Expected Results

Normal healthy individuals should be free of adenovirus and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of adenovirus antigen. Incidence of adenovirus infection varies significantly between populations, season of the year, and geographic regions. No expected prevalence level can be assumed.

Performance Characteristics

Study #1 – vs.
EM N = 118

		EM	
		+	-
DAI	+	31	6
	-	0	81

Sensitivity: 100% (31/31)
Specificity: 93.1% (81/87)

Study #2 – vs. another ELISA
N = 116

		Other ELISA	
		+	-
DAI	+	6	2
	-	0	108

Positive Agreement: 100% (6/6)
Negative Agreement: 98.2% (108/110)

Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

Troubleshooting

Problem: Negative control has substantial color development.

Correction: Washings were insufficient. Repeat test with more vigorous washings.

References

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Date Adopted	2016-05-18
REF 8300-3	DA-Adenovirus



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ISO 13485-2003



Revision Date: 2005-07-29