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IVD	See external label	2°C 8°C	96 tests	REF 8300-3

Adenovirus (Fecal)

REF **8300-3**

Cat # Number	**8300-3**
Test	**Adenovirus ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**ELISA - Sandwich; Antibody Coated Plate**
Detection Range	**Qualitative Positive; Negative control**
Sample	**1 gm stool sample**
Specificity	**93.1%**
Sensitivity	**100%**
Total Time	**~ 100 min**
Shelf Life	**24 Months from the manufacturing date**

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

Intended Use

The DAI 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 prolifering 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.

Materials Provided

Adenovirus Stool Antigen Microwell ELISA Kit

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 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 5 % phosphoric acid.	SOLN

Materials Required But Not Provided

Pipettes
Squeeze bottle for washing strips
Reagent grade (DI) water
Graduated cylinder
Sample dilution tubes
ELISA plate reader with 450 and 620-650 nm filters (optional if results are read visually).

Precautions

1. Do not deviate from the specified procedures when performing this assay.
2. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
3. For In Vitro Diagnostic Use Only.
4. Do not interchange reagents between kits with different lot numbers.
5. Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
6. Unused microwells should be stored in the desiccated pouch to protect them from moisture
7. Do not use solutions if they precipitate or become cloudy.
   Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
8. Do not add azides to the samples or any of the reagents.
9. Controls and some reagents contain Thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
10. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
11. Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.
12. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

Storage Conditions

Reagents, strips and bottled components:

Store between 2 – 8 ºC.
Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25ºC)

Preparation

Before use, bring all reagents and samples to room temperature (15-25˚C) and mix.

Wash concentrate (20X) may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25˚C) and mixed. **Ensure that wash concentrate (20X) is completely in solution before diluting to working concentration**. To dilute wash concentrate (20X) to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

Test Samples

Collection of Stool (Feces)

Stools should be collected in clean containers.
Samples should be kept at 4 ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ºC until used. Freezing the specimens does not adversely affect the test; however, avoid repeated freeze/thaw cycles.
All dilutions must be made with the diluted wash buffer.

Preparation of Sample

Fresh/Frozen Stools

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 formalin fix samples prior to testing.

Procedure

Notes:

- Ensure all samples and reagents are at room temperature (15-25˚C) before use. Frozen samples must be thawed completely before use.
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help minimize bubbles in the wells.
- Controls must be included each time the kit is run. Controls are provided prediluted; hence, DO NOT dilute further.

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. *After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 5 minutes, then wash.*After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 5 minutes, then wash.*After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
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 an ELISA plate reader 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, decanting out the contents and refilling the wells for a total of 3 times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

Interpretation of Results - Visual

Reactive: Any sample well that is obviously more yellow than the negative control well.
Non-reactive: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result.

Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using a dichromatic 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.
- DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
- A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for Adenovirus.

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 – 31/31 = 100%
Specificity – 81/87 = 93.1%

Study #2 – vs. another ELISA
N = 116

		Other ELISA	
		+	-
DAI	+	6	2
	-	0	108

Sensitivity – 6/6 = 100%
Specificity – 108/110 = 98.2%

Quality Control

The positive and negative control must be included each time the kit is run. The use of a positive and negative control allows easy validation of kit stability.

- Negative control should appear colorless to faintly yellow when read visually and should read less than 0.15 OD when read at a dual wavelength of 450/620-650 nm.
- Positive control should be a clearly visible yellow color and read greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

Troubleshooting

Problem: Negative control has substantial color development.
Reason*:* Inadequate washings
Correction: Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out.

References

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Date Adopted	**2016-11-17**
REF **8300-3**	**DA-Adenovirus (Fecal)**

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Revision 02