

Adenovirus ELISA Kit

Immunoassay for qualitative detection of adenovirus antigen in feces

Catalog #:CT9002B

Intended Use

The Adenovirus ELISA is an *in vitro* procedure for the qualitative determination of adenovirus antigen in feces. It is a double antibody (sandwich) ELISA using a polyclonal anti-adenovirus antibody to capture the antigen from the stool supernatant. A second anti-adenovirus monoclonal antibody is then added, which binds to the complex. This reaction is visualized by the addition of anti-mouse antibodies conjugated to peroxidase. The resulting blue color, following the addition of the chromogen and peroxide, indicates the presence of adenovirus antigens being bound by the anti-adenovirus antibodies.

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, laborintensive, 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.

REAGENTS AND MATERIALS

1. Microwells: anti-adenovirus polyclonal antibodies
2. Reagent 1: 11 ml anti-adenovirus monoclonal antibodies
3. Reagent 2: 11 ml anti-mouse antibodies conjugated to HRP
4. Positive Control: 1ml diluted adenovirus antigen in buffer
5. Negative Control: 1ml buffer
6. Substrate Solution A: 6ml Urea Peroxide
7. Substrate Solution B: 6ml TMB
8. Stop Solution: 6ml 2M acid sulphur.

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Handle all specimens as if they contain infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving for at least one hour. Alternatively, treat with a 0.5 or 1% solution of sodium hypochlorite for one hour before disposal.
3. Wear protective clothing (laboratory coats and disposable gloves) when assaying samples.
4. Do not eat, drink or smoke in areas where specimens and kit reagents are handled.
5. Avoid contact between hands and eyes or nose during specimen collection and testing.

SPECIMEN COLLECTION

Stool samples must be taken as soon as the symptoms appear. Viral particles decrease in number after one week, making the diagnosis more difficult. The samples can be stored in the refrigerator for 1 to 2 days. For longer storage they must be kept frozen at -20°C. In this case, the sample should be totally thawed,

and brought to room temperature and homogenised before testing.

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).

STORAGE OF TEST KIT

The Adenovirus Rapid Test Strip can be stored at any temperature between 2-8°C. **Do not freeze.** The stability of the kit under these storage conditions is 12 months. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURES

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 1 drop Substrate Solution A and Substrate Solution B 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.

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.

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.

LIMITATIONS OF THE ASSAY

1. The test should be used only for the detection of adenovirus antigen in faecal samples.
2. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line, when reporting the result
3. More than 1600 samples were evaluated to assure the correct performance of the test. The correlation of the results with other techniques (ELISA) was excellent. However, interferences in the performance of the tests should not be excluded.
4. No cross-reactions with other viruses or substances were observed during the evaluation of the test. A negative result does not totally exclude a possible rotavirus infection. The significance of the results must be evaluated in relation to the patient's clinical symptoms.

PERFORMANCE

		Biocare Adeno	
		Positive	Negative
IVD Adenovirus ELISA	Positive	152	3
	Negative	2	1402

Specificity: $1402/(1402+2)=99.8\%$

Sensitivity: $152/(152+3)=98\%$

Inter-series and intra-series accuracy: 100 %

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