Cryptosporidium (Fecal) ELISA Kit

Cat. No.:DEIA2436
Pkg.Size:96T

Intended use

This ELISA is an in immunoassay for the qualitative determination of Cryptosporidium antigen in feces.

Introduction

Cryptosporidium is a coccidian parasite that is recognized as an important enteric pathogen. The organism causes an acute, though self-limiting infection in immunocompetent individuals. Incubation periods of 1 to 12 days have been reported with most oocyst shedding ending by day 21. Symptoms range from mild to severe diarrhea with a variety of complications. The infection in immunocompromised patients is much more severe and may often be life threatening. Passage of fluid, up to 12 liters per day, has been reported. Multiple pathways of Cryptosporidium transmission have been implicated. These include animal to human, water contamination and person-to-person. The latter may include contact between members of the same household, day care centers, and homosexual men. Diagnosis of Cryptosporidium infections was done originally by direct detection techniques. Of these, microscopic examination of stools using stains or fluorescence labeled antibodies has been the most common. However, this method relies on an experienced technician and subsequent observation of intact organisms. Because of the historically low proficiency of correct microscopic examinations, alternative diagnostic methods have been investigated. One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with stools. These tests, which have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms.

Principle Of The Test

During the first incubation, Cryptosporidium antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-Cryptosporidium antibody that "sandwiches" the antigen. The next incubation adds an anti-second antibody conjugated to peroxidase. 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 Provided

1. Test Strips Microwells containing anti-Cryptosporidium polyclonal antibodies - 96 test wells in a test strip holder.
2. Enzyme Conjugate: One bottle containing 11 mL of goat anti- Cryptosporidium antibodies with blue dye and Thimerosal.
3. Positive Control: One vial containing 2 mL of a diluted Cryptosporidium positive formalinized stool supernatant.
4. Negative Control: One vial containing 2 mL of a Cryptosporidium negative formalinized stool supernatant.
5. Chromogen: One bottle Containing 11 mL of the chromogen tetramethylbenzidine (TMB) and peroxide.
5. Wash Concentrate (20X): Two bottles containing 25 mL of concentrated buffer and surfactant with Thimerosal.
6. Dilution Buffer: Four bottles containting 30 mL buffered protein solution containing Thimerosal
7. Stop Solution: One bottle containing 11 mL of 5% phosphoric acid.

Materials Required But Not Supplied

1. Transfer Pipettes

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2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Graduated Cylinder
4. Reagent grade (DI) water

Storage

Store between 2 – 8 ºC.

Specimen Collection And Handling

Collection of Stool (Feces)

Stool samples may be used as unpreserved or frozen, or in preservation media of 10% formalin, SAF or MF. Unpreserved samples should be kept at 2 – 8 ºC and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ºC or lower until used. Freezing does not adversely affect the test. Formalized, SAF and MF preserved samples may be kept at room temperature (15-25 ºC) and tested within 18 months of collection. DO NOT freeze preserved samples. All dilutions of unpreserved stools must be made with the wash buffer.

Assay Steps

In Well Dilution Procedure:

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Prepare sample dilutions in tubes using 0.3 mL of Dilution Buffer and 0.1 g, about the size of a small pea, of fecal sample using an applicator stick. Mix thoroughly before using. -IF USING SWABS, add 0.6 mL of dilution buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.
3. For watery unpreserved specimens, mix contents then add 0.1 mL of sample to 0.3 mL Dilution Buffer in dilution tubes. Mix thoroughly before using.
4. For samples in SAF, 10% Formalin or Cary-Blair, mix contents thoroughly inside container. No further processing is required.
5. Using a micropipette, add 100 µl of negative control to well # 1 and 100 µl of positive control to well # 2.
6. Using a micropipette, add 50 µl of Dilution Buffer to each sample well. DO NOT add Dilution Buffer to control wells.
7. Add 50 µl of sample to each sample well with Dilution Buffer.
8. Incubate for 60 minutes at room temperature (15-25℃), then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer. Proceed to step 9.

In Tube Dilution Procedure:

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Prepare sample dilutions in tubes using 0.7 mL of Dilution Buffer and 0.1 g, about the size of a small pea, of fecal sample using an applicator stick. Mix thoroughly before using.
-IF USING SWABS, add 1 mL of dilution buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.
3. For watery unpreserved specimens, mix contents then add 0.1 mL of sample to 0.7 mL of Dilution Buffer in dilution tubes. Mix thoroughly before using.
4. For samples in SAF, 10% Formalin or Cary-Blair, mix contents then add 0.2 mL of sample to 0.3 mL of Dilution Buffer in dilution tubes. Mix thoroughly before using.
5. Using a micropipette, add 100 µl of negative control to well # 1.
6. Using a micropipette, add 100 µl of positive control to well # 2.
7. Add 100 µl of diluted sample to each well.
8. Incubate for 60 minutes at room temperature (15-25℃), then wash.* After last wash, slap the wells out on a clean absorbent

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towel to remove excess wash buffer. Proceed to step 9.
9. Add 2 drops of Enzyme Conjugate to each well.
10. Incubate for 30 minutes at room temperature (15-25℃), then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
11. Add 2 drops of Chromogen to each well.
12. Incubate for 10 minutes at room temperature (15-25℃).
13. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately 15 seconds. Read reaction within 5 minutes after adding stop solution.
14. Read results visually or using an ELISA plate reader (see instructions below).
* Washings consist of vigorously filling each well to overflowing and decanting contents five separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an absorbance of at least 0.5 OD units and the negative control must be less than 0.15 OD units. Should the value fall below this limit, the kit should not be used.

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.
ELISA Reader
Zero reader on air. Read all wells at 450/620-650 nm.
Reactive: Absorbance reading of 0.15 OD units and above indicates the sample contains Cryptosporidium antigen.
Non-reactive: Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of Cryptosporidium antigen.

Sensitivity

This assay can detect approximately 30 nanograms per mL of Cryptosporidium antigen.

Precautions

1. Do not use solutions if they precipitate or become cloudy. Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
2. Do not add azides to the samples or any of the reagents.
3. Controls and some reagents contain Thimerosal as a preservative.
4. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.

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

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suspected of being positive for Cryptosporidium.

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