DRG® Cryptosporidium Ag (stool) (EIA-3467)

IVD

CE

DIRECTIONS FOR USE
For In Vitro Diagnostic Use.

INTENDED USE

This ELISA is an *in vitro* immunoassay for the qualitative determination of *Cryptosporidium* antigen in feces. It is a double antibody (sandwich) ELISA using an anti-*Cryptosporidium* antibody to capture the antigen from the stool supernatant. A second anti-*Cryptosporidium* antibody is then added which sandwiches the captured antigen. This reaction is visualized by the addition of an anti-second antibody conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *Cryptosporidium* antigens being bound by the anti-*Cryptosporidium* antibodies.

SUMMARY

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. [1,8,9,10,11,13]

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. [1,2,3,12,14,16]

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. [1,2,12,14,16]

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. [4,5,16,17]

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.[6,7]

PRINCIPLE OF PROCEDURE

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

- Test strips: microwells containing anti-*Cryptosporidium* polyclonal antibodies - 96 test wells.
- Test strip holder: One (1).
- Reagent 1: One (1) bottle containing 11 ml of goat *anti-Cryptosporidium* antibodies with blue dye and Thimerosal.
- Reagent 2: One (1) bottle containing 11 ml of anti-goat-peroxidase with red dye and Thimerosal.

- Positive control: One (1) vial containing 1 ml of a diluted *Cryptosporidium* positive formalinized stool supernatant.
- Negative control: One (1) vial containing 1 ml of a *Cryptosporidium* negative formalinized stool supernatant.
- Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.
- Wash Concentrate 20X: Two (2) bottles containing 25 ml of concentrated buffer and surfactant with Thimerosal.
- Stop solution: One (1) bottle containing 11 ml of 1M phosphoric acid.

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.

STORAGE CONDITIONS

Reagents, strips and bottled components:

- Store between 2 - 8ºC.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

PREPARATION

Wash Buffer – 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.

Collection of Stool (Feces)

No modification of collection techniques used for standard microscopic O&P examinations is needed. 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 - 8º C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20° C or lower until used. Freezing does not adversely affect the test.
Formalized, SAF and MF preserved samples may be kept at room temperature (15-25º C) and tested within 18 months of collection. DO NOT freeze preserved samples.
All dilutions of unpreserved stools must be made with the wash buffer.

PREPARATION OF SAMPLE:

Fresh/Frozen Stools – Thaw sample if needed. Add sufficient diluted wash buffer to make approximately a 1:4 dilution (1 gram or a pea size of fecal sample to 3 ml of diluted wash buffer) and mix well.

Preserved Stools (Formalin, SAF and MF) – Mix contents thoroughly inside collection container. No further processing is required.

PROCEDURE

Materials Provided

Cryptosporidium Stool Antigen Microwell ELISA Kit

Materials Required But Not Provided

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

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters (DRG ELM3200 or ELM5000)

NOTE: All incubations are at room temperature (15 to 25º C)

TEST PROCEDURE

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 2 drops (approximately 100 ul) of negative control to well # 1 and 2 drops of positive control to well # 2.
3. Add 2 drops of the stool supernatant to each test well.
4. Incubate for 30 minutes at room temperature (15-25° C), then wash.*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate for 5 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate for 5 minutes, then wash.
9. Add 2 drops of Chromogen to each well.
10. Incubate 5 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
12. Read results visually or at 450/620-650 nm. Zero reader on air.

* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times.

Controls must be included each time the kit is run.

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 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.

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 *Cryptosporidium*.

EXPECTED RESULTS

Normal healthy individuals should be free of *Cryptosporidium* and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of *Cryptosporidium* antigen. Certain populations, such as homosexual men and children in day care settings, have shown higher rates of infection with *Cryptosporidium* than the normal population. Please refer to the Summary section for references.

PERFORMANCE CHARACTERISTICS

Study #1 – vs. Microscopy
N = 71

	Micro +	**Micro -**
DRG-Novum ELISA +	25	1
DRG-Novum ELISA -	2	43

Sensitivity – 25/27 = 93%
Specificity – 43/44 = 98%

Analytical Sensitivity

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

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.

TROUBLESHOOTING

Problem: Negative control has substantial color development.
Correction: Washings were insufficient. Repeat test with more vigorous washings

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