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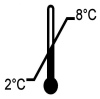
IVD  See external label   96 tests REF 8310-3

Crypto / Giardia Ag Combo (Fecal) ELISA

Cat # 8310-3

Test	**Crypto/ Giardia Combo ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**Sandwich ELISA: Antibody Coated Plate**
Detection Range	**Qualitative - Positive, Negative and Cut-off**
Sample	**Stool sample**
Specificity	**100%**
Sensitivity	**99%**
Total Time	**~100 min**
Shelf Life	**12 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

This ELISA is an *in vitro* immunoassay for the qualitative determination of *Giardia* and *Cryptosporidium* antigen in feces.

SUMMARY AND EXPLANATION

Giardia lamblia is the protozoan parasite responsible for the disease giardiasis. Symptoms of acute giardiasis include diarrhea, nausea, weight loss, malabsorption, abdominal cramps, flatulence and anemia. The disease may manifest itself as an acute, chronic or as an asymptomatic infection. Giardiasis is the most prevalent parasitic disease in the United States and is responsible for an estimated 100 million mild infections and 1 million severe infections each year.

The mode of transmission of *Giardia* is through fecal-oral ingestion of cysts. Epidemics of giardiasis have been documented in day care centers and by drinking contaminated water. Day care centers may be directly or indirectly responsible for 45% of diagnosed *Giardia* infections in the United States. One study found 54% of the children at a day care center were infected.

Another important source of *Giardia* infection is among homosexual men. Prevalence rates of 5 to 19% for this population have been reported.

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 *Giardia* and *Cryptosporidium* infections has been done through a number of invasive and non-invasive techniques. Of the non-invasive techniques, microscopic examination of stools 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 and intermittent excretion of organisms, 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 have shown comparable sensitivity to experienced microscopic examinations, are fairly simple to perform and do not require the observation of intact organisms.

PRINCIPLE OF PROCEDURE

During the first incubation, *Giardia* and/or *Cryptosporidium* antigens present in stool specimens are captured by antibodies attached to the microwells. The wells are incubated and washed before anti-*Giardia* and anti-*Cryptosporidium* antibodies conjugated to peroxidase are added. The enzyme conjugate will “sandwich” any antigen bound to the wells. After washings to remove any unbound enzymes, a Chromogen is added, which develops a blue color in the presence of the enzyme complex. The Stop Solution ends the reaction and turns the blue color to yellow. If no antigen is captured, or if there is an insufficient level of antigen, no colored reaction will take place.

REAGENTS

Item	Description	Symbol
Test Strips	Microwells containing anti-*Giardia* and anti-*Cryptosporidium* antibodies - 96 test wells in a strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-Giardia and anti-Cryptosporidium antibodies conjugated to horseradish peroxidase with Thimerosal.	CONJ
Giardia Positive Control	One (1) vial containing 2 ml of a diluted *Giardia* positive formalinized stool supernatant.	CONTROL +
Cryptosporidium Positive Control	One (1) vial containing 2 ml of a diluted *Cryptosporidium* positive formalinized stool supernatant	CONTROL +
Negative Control	One (1) vial containing 2 ml of buffered protein solution with Thimerosal.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	SUBS TMB
Dilution Buffer	Four (4) bottles containing 30 ml of a buffered protein solution with Thimerosal.	SPECM DIL
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer with surfactant and Thimerosal.	WASH BUF
Stop Solution	One (1) bottle containing 11 ml of 5% phosphoric acid.	SOLN

WARNINGS/PRECAUTIONS

• Do not deviate from the specified procedures when performing this assay. 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.
• For In Vitro Diagnostic Use Only.
• Do not interchange reagents between kits with different lot numbers.
• 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.
• Unused microwells should be stored in the desiccated pouch to protect them from moisture.
• 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, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
• Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
• 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.
• 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).

PREPARATIONS

1. Before use, bring all reagents and samples to room temperature (15-25 ℃) and mix.
2. (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25℃) and mixe d. **Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration.** To dilute (20X) wash concentrate 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.

COLLECTION OF STOOL (FECES)

1. No modification of collection techniques used for standard microscopic O&P examinations is needed.
2. Stool samples may be used as unpreserved or frozen, in Cary-Blair Transport Medium or in preservation media of 10% formalin or SAF.
3. Unpreserved samples should be kept at 2-8 ℃ and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 ℃ or lower until used. Avoid multiple freeze/thaw cycles.
4. Formalinized and SAF preserved samples may be kept at room temperature (15-25 ℃) or at 2-8 ℃ and tested within 18 months of collection. DO NO T freeze preserved samples.
5. Samples in Cary-Blair should be kept at 2-8 ℃ o r -20 ℃ and tested within 1 week of collection. Avoid multiple freeze/thaw cycles.

Procedure Notes

1. All incubations are to be done at room temperature (15 to 25 ℃)
2. Ensure all samples and reagents are at room temperature (15-25 ℃) before use. Frozen samples must be thawed completely before use.
3. All dilutions of stools must be made with the Dilution Buffer provided. Do not use dilution buffer from a kit with a different lot number.
4. If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.
5. 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 to minimize bubbles in the wells.
6. Controls must be included each time the kit is run. Controls are provided ready to use. DO NOT dilute further.
7. **Unpreserved and Preserved specimens have different testing procedures. See below for specific instructions on how to run the assay using each procedure.**

PROCEDURE

Materials Provided
Giardia/*Cryptosporidium* Stool Antigen Microwell ELISA Kit

Materials Required But Not Provided

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent grade (DI) water
- Micropipette
- Applicator sticks (recommended) or swabs for sample preparation
- Sample dilution tubes

Suggested Equipment

ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

Proper Temperature
All incubations are at room temperature (15 to 25 ºC)

TEST PROCEDURE

Preserved Specimen Procedure

1. **For samples in SAF, 10% Formalin or Cary-Blair**, mix contents thoroughly inside container. No further processing is required.
2. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
3. Using a micropipette, add **100 µl** of negative control to well # 1 and **100 µl** of positive control to well # 2.
4. Using a micropipette, add **50 µl** of Dilution Buffer to each sample well. **DO NOT add Dilution Buffer to control wells.**
5. Add **50 µl** of sample to each sample well with Dilution Buffer.
6. 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.
7. Add **2 drops** of Enzyme Conjugate to each well.
8. 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.
9. Add **2 drops** of Chromogen to each well.
10. Incubate for **10 minutes** at room temperature (15-25℃).
11. 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.
12. Read results visually or using an ELISA plate reader (see instructions below).

Unpreserved Specimen Procedure

1. 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
2. **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.
3. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder.
4. Using a micropipette, add **100 µl** of negative control to well # 1.

5. Using a micropipette, add **100 µl** of positive control to well # 2.
6. Add **100 µl** of diluted sample to each well.
7. 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.
8. Add **2 drops** of Enzyme Conjugate to each well.
9. 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.
10. Add **2 drops** of Chromogen to each well.
11. Incubate for **10 minutes** at room temperature (15-25℃).
12. 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.
13. 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 (5) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.**

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 bichromatic reading with filters at 450 nm and 620-650 nm.**
Reactive: Absorbance reading of 0.08 OD units and above indicates the sample contains *Giardia* or *Cryptosporidium* antigen.
Non-reactive: Absorbance reading less than 0.08 OD units indicates the sample does not contain detectable levels of *Giardia* or *Cryptosporidium* antigen.

LIMITATION OF PROCEDURE

- 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 *Giardia* or *Cryptosporidium*.

EXPECTED VALUES

Normal healthy individuals should be free of *Giardia* and *Cryptosporidium* and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of either antigen.

SPECIFIC PERFORMANCE CHARACTERISTICS

Study #1
A study was performed with the Diagnostic Automation, Inc. G/C Combo assay using 135 fresh/frozen specimens confirmed positive or negative by microscopy. Of the 135 specimens 12 were confirmed positive for either Cryptosporidium or Giardia, and 123 specimens were confirmed negative for both Cryptosporidium and Giardia. The results from the study are shown in the table below.

Microscopy			
		DAI	
		+	-
Reference Method*	+	12	3
	-	0	120

Positive Agreement: 100% (12/12)
Negative Agreement: 97.6% (120/123)

Study # 2

Giardia			
		DAI	
		+	-
Reference Method*	+	35	0
	-	0	95

Positive Agreement: 100% (35/35)
Negative Agreement: 100% (95/95)

Cryptosporidium			
		DAI	
		+	-
Reference Method*	+	52	0
	-	1	95

Positive Agreement: 100% (52/52)
Negative Agreement: 99% (95/96)

Cryptosporidium/Giardia			
		DIA	
		+	-
Reference Method*	+	11	0
	-	1	95

Positive Agreement: 100% (11/11)
Negative Agreement: 99% (95/96)

*Reference Method refers to a commercially available ELISA.

QUALITY CONTROL

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

• Negative control should appear colorless when read visually and should read less than 0.08 OD when read at a dual wavelength of 450/620-650 nm.
• Positive controls should each be a clearly visible yellow color and read at greater than 0.5 OD when read at a dual wavelength of 450/620-650 nm.

REPRODUCIBILITY

- The intra-assay (well to well) CV was calculated using 3 Cryptosporidium positive, 3 Giardia positive and 4 Giardia/Cryptosporidium negative samples assayed 10 times in a single run. The mean CV for all samples was 11.8%.

- The inter-assay (run to run) CV was calculated using 3 Cryptosporidium positive, 3 Giardia positive and 4 Giardia/Cryptosporidium negative samples assayed on three separate days. The mean CV for all samples was 7.5%

TROUBLESHOOTING

- **Problem:** Negative control has excessive color after 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.

CROSS REACTIVITY

No cross-reactions were seen with the following organisms:
Entamoeba hartmanni, Endolimax nana, Entamoeba histolytica/dispar, Entamoeba coli, Blastocystis hominis, Dientamoeba fragilis, *Chilomastix mesnili, Strongyloides stercoralis, Ascaris lumbricoides, Enterobius vermicularis, Diphyllobothrium* species, *Hymenolepis nana, Clonorchis sinensis, Enteromonas hominis, Trichuris trichiura, Iodamoeba buetschlii,* Hookworm, *Schistosoma mansoni,* rotavirus, *Taenia* eggs, *Fasciola* eggs, *Isospora belli, Entamoeba polecki*, adenovirus*, &* 33 bacterial species (list available on request).

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