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**ISO 13485-2003**

IVD



See external label



2°C-8°C



Σ=96 Tests

REF

8304-3

# *Giardia lamblia* Antigen Detection ELISA

DOUBLE ANTIBODY SANDWICH PRINCIPLE

Cat. No. 8304-3

## INTENDED USE

This ELISA is an *in vitro* immunoassay for the qualitative determination of *Giardia* antigen in feces. It is a double antibody (sandwich) ELISA using an anti-*Giardia* antibody to capture the antigen from the stool supernatant. A second anti-*Giardia* 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 *Giardia* antigens being bound by the anti-*Giardia* antibodies.

## 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.<sup>1</sup> 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.<sup>9</sup>

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.<sup>1,2</sup> Day care centers may be directly or indirectly responsible for 45% of diagnosed *Giardia* infections in the United States.<sup>4</sup> One study found 54% of the children at a day care center were infected.<sup>1</sup>

Another important source of *Giardia* infection is among homosexual men. Prevalence rates of 5 to 19% for this population have been reported.<sup>8</sup>

Diagnosis of giardiasis 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.<sup>3,5,6,10,11</sup>

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.<sup>5,6,7,12</sup>

## PRINCIPLE OF PROCEDURE

During the first incubation, *Giardia* antigens present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds an additional anti-*Giardia* 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-*Giardia* polyclonal antibodies - 96 test wells.
- Test strip holder: One (1).
- Reagent 1: One (1) bottle containing 11 ml of goat anti-*Giardia* polyclonal 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 *Giardia* positive formalinized stool supernatant.
- Negative control: One (1) vial containing 1 ml of a *Giardia* 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.

## **WARNINGS/PRECAUTIONS**

- For In Vitro Diagnostic Use
- 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.
- 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.
- Use of SAF preserved specimens is contra-indicated in this ELISA.
- 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°C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

## **SPECIMEN COLLECTION AND PREPARATION**

- **Collection of Stool (Feces)**
  1. 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.
  2. Unpreserved samples should be kept at 2 - 8° C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -15° to -25°C or lower until used. Freezing does not adversely affect the test.
  3. Formalized preserved samples may be kept at room temperature (15-25° C) and tested within 18 months of collection. DO NOT freeze preserved samples.
  4. All dilutions of unpreserved stools must be made with diluted wash buffer.
  5. Wash Buffer Preparation – 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.
- **Preparation of Fresh/Frozen Stools**
  1. 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.
- **Preparation of Preserved Stools (Formalin)**
  1. Mix contents thoroughly inside collection container. No further processing is required.

## **MATERIALS PROVIDED**

*Giardia* 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

### **Suggested Equipment**

ELISA plate reader with 450 and 620-650 nm filters

## **Proper Temperature**

All incubations are at room temperature (15 to 25° C)

## **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.

## **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. Please refer to the enclosed visual read card for color comparisons.

### **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 *Giardia* antigen.

**Non-reactive:** Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of *Giardia* antigen.

### **Analytical Sensitivity**

This assay can detect approximately 5-10 nanograms per ml of *Giardia* 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.

## **EXPECTED VALUES**

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

## Specific Performance Characteristics

### Study #1

A total of 186 formalin preserved stools (40 positive and 146 negative stools as determined by microscopy by a USA government laboratory) were tested against the ELISA test. The following results were obtained.

	Micro +	Micro -
ELISA +	34	6
ELISA -	6	140

Sensitivity: 85.0% 95% CI = 70.2% to 94.3%

Specificity: 95.9% 95% CI = 91.3% to 98.5%

### Study #2

A total of 63 unpreserved stools (10 positive and 53 negative stools as determined by microscopy in an independent clinical laboratory) were tested against the ELISA test.

The following results were obtained.

	Micro +	Micro -
ELISA +	10	0
ELISA -	0	53

Sensitivity: 100% 95% CI = 69.2% to 100%

Specificity: 100% 95% CI = 93.3% to 100%

### Study #3

A total of 85 formalin preserved stools were tested with the DAI ELISA test and another manufacturer's ELISA kit.

	OTHER ELISA +	OTHER ELISA -
DAI +	11	3
DAI -	1	70

% Positive Agreement = 92% (11/12)

% Negative Agreement = 90% (70/73)

### Analytical Sensitivity

This assay can detect approximately 5-10 nanograms per ml of *Giardia* antigen.

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*, *Cryptosporidium*, *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).

### Summary of Reproducibility Data

Fourteen samples plus kit controls were run at 3 sites for 3 days. The 7 negative samples had a mean range of -0.001 to .005 (SD range of .006 to .015). The 4 low positive samples had a mean range of .229 to .375 (SD range of .055 to .076). The 3 high positive samples had a mean range of .650 to 1.165 (SD of .116 to .136).

## TROUBLESHOOTING

**Problem:** Negative control has substantial color development.

**Correction:** Washings were insufficient. Repeat test with more vigorous washings

## 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*.

## VALIDITY

**Please do not use this kit beyond the expiration indicated on the kit box and reagent labels.**

### References:

1. Black, R. et. al.: Giardiasis in Day-Care Center: Evidence of Person to Person Transmission. *Pediatric*, #60 (4), October 1977, pp. 486-491.
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7. Green, E. Miles, M., and Warhurst, D.: Immunodiagnostic Detection of *Giardia* Antigen in Faeces by a Rapid Visual Enzyme Linked Immunosorbent Assay. *Lancet*, Sept. 28, 1985, pp. 691-693.
8. Peters, C. et al.: Prevalence of Enteric Parasites in Homosexual Patients Attending an Outpatient Clinic. *J Clin Micro*, Oct. 1986, Vol. 24 (4), pp. 684-685.
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10. Burke, J.: Giardiasis in Childhood. *Am J Dis Child*, Nov. 1975, Vol. 129, pp. 1304-1310.
11. Danciger, M. and Lopez, M.: Numbers of *Giardia* in the Feces of Infected Children. *Am J Trop Med Hyg.*, Vol. 24 (2), 1975, pp. 237-242.
12. Ungar, B. et. al: Enzyme Linked Immunosorbent Assay for the Detection of *Giardia Lambli*a in Fecal Specimens. *J Infect Dis.*, January 1984, Vol. 149 (1), pp. 90-97.



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