AccuDiag™ Giardia ELISA Kit

REF 8304-3

IVD See External Label 2°C 8°C 96 Tests

Giardia ELISA	
Principle	Sandwich Complex ELISA
Detection	Qualitative
Incubation Time	100 minutes
Sensitivity	100%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

DAI ELISA is an *in vitro* immunoassay for the qualitative determination of *Giardia* antigen in fecal specimens.

SIGNIFICANCE AND SUMMARY

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.[9]

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

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.[3,5,6,10,11]
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.[5,6,7,12]

ASSAY PRINCIPLE

During the first incubation, *Giardia* specific antigen present in the stool specimens are captured by monoclonal antibodies attached to the microwells. The wells are incubated and washed before anti-*Giardia* polyclonal antibodies conjugated to horseradish peroxidase are added. The enzyme conjugate will "sandwich" any antigen bound to the wells. After washings to remove unbound enzyme, 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.

SPECIMEN COLLECTION AND PREPARATION

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°C and tested within 24 hrs of collection. Samples that cannot be tested within this time should be frozen at -20°C or lower until used. Avoid multiple freeze/thaw cycles.
4. Formalinized and SAF preserved samples may be kept at room temperature (15-25°C) or at 2-8°C and tested within 18 months of collection. DO NOT freeze preserved samples.
5. Samples in Cary-Blair should be kept at 2-8°C or -20°C and tested within 1 week of collection. Avoid multiple freeze/thaw cycles.

REAGENTS

Materials provided with the kit

Giardia Stool Antigen Detection Microwell ELISA Kit

1. **Test Strips:** Microwells containing anti-*Giardia* monoclonal antibodies: 96 test wells in a test strip holder.
2. **Enzyme Conjugate:** One (1) bottle containing 11 ml anti-*Giardia* polyclonal antibodies conjugated to horseradish peroxidase with preservative.
3. **Positive Control:** One (1) vial containing 2 ml of a diluted *Giardia* positive antigen formalinized stool supernatant.
4. **Negative Control**: One (1) vial containing 2 ml of dilution buffer.
5. **Chromogen:** One (1) bottle containing 11 ml of tetramethylbenzidine (TMB) and peroxide.
6. **Wash Concentrate (20X):** Two (2) bottles containing 25 ml of concentrated buffer with detergent and thimerosal.
7. **Dilution Buffer:** Four (4) bottles containing 30 ml of a buffered protein solution with thimerosal.

8. **Stop Solution:** One (1) bottle containing 11 ml of 5% phosphoric acid solution.

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
5. Micropipette
6. Applicator sticks (recommended) or swabs for sample preparation.
7. Sample dilution tubes
8. ELISA plate reader capable of reading bichromatically at 450/620-650 nm.

REAGENTS PREPARATION

1. Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
2. (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. **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

ASSAY PROCEDURE

Preserved Specimen

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°C), 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°C), 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°C).
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°C), 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°C), 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°C).
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.**

Procedural Notes

1. All incubations are to be done at room temperature (15 to 25°C)
2. Ensure all samples and reagents are at room temperature (15-25°C) 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.**

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.

RESULTS AND INTERPRETATION

Interpretation of Results - Visual

1. **Positive:** Any sample well that is obviously more yellow than the negative control well.
2. **Negative:** 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

1. Zero reader on air. ***Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.***
2. **Positive:** Absorbance reading of 0.08 OD and above indicates the sample contains *Giardia* antigen.
3. **Negative:** Absorbance reading less than 0.08 OD indicates the sample does not contain detectable levels of *Giardia* antigen.

QUALITY CONTROL

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.
Expected values for the controls are when read at a dual wavelength of 450/620-650:

Negative - 0.0 to 0.8 OD units
Positive - 0.5 OD units and above

EXPECTED VALUES

1. Normal healthy individuals should be free of *Giardia* and should test negative.
2. A positive reaction indicates that the patient is shedding detectable amounts of *Giardia* antigen.
3. Certain populations, such as 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.

PERFORMANCE CHARACTERISTICS

Study #1 A study was performed with the Diagnostic Automation, Inc. *Giardia* assay using fresh/frozen specimens, specimens preserved in 10% Formalin and SAF and specimens in Carey-Blair Transport Media. There were a total of 90 specimens used in the study that were identified positive or negative for *Giardia* by microscopy. Of the 90 specimens, 26 were determined to be positive for *Giardia* and 64 were negative for *Giardia*. The results from the study are shown in the following table.

Diagnostic Automation, Inc. *Giardia*		Microscopy	
		+	–
	+	26	0
	–	0	64

Sensitivity: 100% (26/26)
Specificity: 100% (64/64)

Study #2 Another study was performed comparing the Diagnostic Automation, Inc. *Giardia* assay with another commercially available ELISA. The study was performed using fresh/frozen specimens and specimens preserved in 10% Formalin and SAF. There were a total of 86 specimens used in the study that were identified either positive or negative for *Giardia* by microscopy. Of the 86 specimens, 22 were identified positive for *Giardia* and 64 were negative for *Giardia*. The results from the study are shown in the following table.

Other Commercial ELISA		Diagnostic Automation, Inc.	
		+	–
	+	22	0
	–	0	64

Positive Agreement: 100% (22/22)
Negative Agreement: 100% (64/64)

Reproducibility

1. The intra-assay (well to well) CV was calculated using 4 positive and 4 negative samples assayed 24 times in a single run. The mean CV was 3.67% with the highest being 6.18 %.
2. The inter-assay (run to run) CV was calculated using 4 positive and 4 negative samples assayed on three separate days. The mean CV was 4.08% with the highest being 11.61%.

Cross Reactivity

No cross-reactions were seen with the following organisms:
Etamoeba 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).

LIMITATIONS OF THE ASSAY

1. Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
2. DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.
3. 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*.

PRECAUTIONS

1. **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.
2. For In Vitro Diagnostic Use Only.
3. Do not interchange reagents between kits with different lot numbers.
4. 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.
5. Unused microwells should be stored in the desiccated pouch to protect them from moisture.
6. Do not use solutions if they precipitate or become cloudy.
   Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
7. Do not add azides to the samples or any of the reagents.
8. 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.
9. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
10. 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.
11. 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

1. Reagents, strips and bottled components should be stored at 2-8°C.

2. Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25°C).

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

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Diagnostic Automation/Cortez Diagnostics, Inc.
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REF 8304-3	**AccuDiag™ - Giardia ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu**
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