Giardia
ELISA [Enzyme-Linked ImmunoSorbent Assay]

REF **7069**

Qualitative assay for the determination of
Giardia antigen in human stool samples

March 2012

BIOMERICA

I. INTENDED USE

The Biomerica *Giardia* ELISA is intended for the qualitative detection of *Giardia* antigen in fecal specimens. This assay is intended for *in vitro* diagnostic use only.

II. SUMMARY AND EXPLANATION

Giardia lamblia is a flagellated protozoan parasite which exists in two forms – a noninfectious trophozoite inhabiting the small intestine, and the highly infectious cyst form. The trophozoite is extremely labile, and survives for only hours outside the body. The cyst form, however, may survive for a matter of days, and is responsible for the disease giardiasis.[1]

Symptoms of giardiasis normally begin one to two weeks after becoming infected. Symptoms include diarrhea, gas or flatulence, stomach or abdominal cramps, upset stomach, nausea, malabsorption, and anemia.[2,3,4] Symptoms may last from two to six weeks, and can lead to weight loss and dehydration. 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.[5]

The mode of transmission of *Giardia* is through fecal-oral ingestion of cysts. *Giardia* has been found in all animal hosts studied, and water and food contaminated by animal fecal activity is the most common route of infection.[6] Among groups with inadequate hygiene, infection can occur directly through the fecal-oral route. This mode of transmission is particularly common among young children as well as among male homosexuals.[2,7,8] Widespread epidemics of giardiasis have been documented in day care centers and by drinking contaminated water.[2,6,9] Day care centers may be directly or indirectly responsible for 45% of diagnosed *Giardia* infections in the United States.[9] One study found 54% of the children at a day care center were infected.[2]

The most common method of diagnosing giardiasis has been microscopic examination of stool specimens. However, this method requires extensive experience and the presence of intact cysts in the feces. Because of the historically low proficiency of correct microscopic examinations and intermittent excretion of organisms, alternative diagnostic methods have been investigated.[10,11,12,13,14.]

One important alternative has been the development of an antigen capture enzyme linked immunosorbent assay (ELISA) for use with fecal specimens. These tests have shown comparable or better sensitivity and specificity to experienced microscopic examinations, are simple to perform and do not require the observation of intact organisms.[11,12,13,14.] Large numbers of specimens can be tested rapidly and objectively, and the procedure is less labor intensive than most microscopy methods.

III. PRINCIPLE OF THE TEST

The Biomerica *Giardia* test is an enzyme immunoassay which detects the presence of *Giardia*-specific antigen (GSA) in stool samples. Antibodies to GSA have been immobilized on breakaway microwells. Diluted patient specimens are added to the microwells along with horseradish peroxidase-conjugated antibodies to GSA. If GSA is present in the sample, it will bind to the detecting antibody and the immobilized antibody to form a complex, which will remain in the microwell after washing to remove unbound enzyme. After washing, a substrate/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.

IV. KIT COMPONENTS

Kit Components	Description	Symbol
Test Strips	96 microtiter wells containing anti-*Giardia* antibodies in a test strip holder.	**PLA**
Enzyme Conjugate	One (1) bottle containing 11 ml of HRP-labeled anti-*Giardia* antibodies with thimerosal.	**ENZ CONJ**
Positive Control	One (1) vial containing 2 ml of a diluted *Giardia* positive stool supernatant in formalin.	**CTRL +**
Negative Control	One (1) vial containing 2 ml of a *Giardia* negative stool supernatant in formalin.	**CTRL -**
Substrate	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.	**SUBS TMB**
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer with detergent and thimerosal.	**BUF WASH 20X**
Dilution Buffer	Four (4) bottles containing 30 ml of a buffered protein solution with thimerosal.	**SPEC DIL**
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	**STOP**

MATERIAL AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader (Optional)
- Microplate washer [if washer is unavailable, manual washing may be acceptable].
- Micropipettes capable of delivering 50 µL and 100 µL.
- Transfer Pipettes.
- Wash Bottle.
- Absorbent Paper.
- Timer
- Reagent grade (DI) water.
- Waste container with disinfectant or biohazard bags.
- Sample Dilution Tubes.
- Parafilm or cover for microwell plate
- Applicator sticks (recommended) or swabs for sample preparation

V. WARNINGS AND PRECAUTIONS FOR USERS

- **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. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- All reagents are for *In Vitro* Diagnostic Use Only.
- Reagents from different kit lots should not be interchanged.
- Do not use reagents that are beyond their expiration dates.
- All reagents should be at room temperature before using.
- If using dropper bottles, hold them vertically to ensure proper drop size.
- Wear gloves when performing the test, and handle specimens and used microwells as if able to transmit infectious agents.
- Unused microwells should be stored in the resealable pouch with dessicant 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.
- 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 large amounts of water.

- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any sample spills.
- Stop solution is a 5% solution of phosphoric acid in water. In case of contact with skin or mucous membranes, flush with water immediately.

VI. SAMPLE COLLECTION AND STORAGE

Standard collection techniques used for fecal specimens for culture can be employed. Stool samples may be used as unpreserved or frozen, in transport medium such as Cary-Blair, or in preservation media such as 10% formalin or Sodium Acetate Formalin (SAF). Unpreserved samples should be stored at 2-8°C and tested within 24 hours of collection. Samples that cannot be tested within this time frame should be frozen at -20°C or lower until used. Avoid multiple freeze/thaw cycles. Formalized and SAF preserved samples may be stored at room temperature (15-25°C) or at 2-8°C and tested within 18 months of collection. **DO NOT FREEZE PRESERVED SAMPLES**. 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.

All dilutions of stools must be made with the Dilution Buffer provided.

VII. REAGENT PREPARATION AND STORAGE

1. All reagents, with the exception of the Wash Concentrate are supplied ready-to-use. All reagents should be stored at 2-8° C. The Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.
2. Before use, bring all reagents and samples to room temperature (15-25°C) and mix.
3. Wash Concentrate: To prepare a 1X wash solution, add contents of one bottle of Wash Concentrate (25ml.) to 475 ml of distilled or deionized water and mix.

VIII. SAMPLE PREPARATION

1. Prepare sample dilutions in tubes using **700 µl** of Dilution Buffer and **0.1 g (100 mg)**, about the size of a small pea (~4mm diameter), of fecal sample using an applicator stick. Mix thoroughly before using.
2. **IF USING SWABS**, add **1ml** 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 **100 µl** of sample to **700 µl** of Dilution Buffer in dilution tubes. Mix thoroughly before using.
4. **For samples in SAF, 10% Formalin or Cary-Blair**, mix contents then add **200 µl** of sample to **300 µl** of Dilution Buffer in dilution tubes. Mix thoroughly before using.

NOTES:
- Ensure all samples and reagents are at room temperature (15-25°C) before use.
- Frozen samples must be thawed completely before use.
- All dilutions must be made with the dilution buffer provided.
- If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.

IX. ASSAY PROCEDURE

NOTES:
- All incubations are at room temperature (15-25°C)
- When running the assay, try to avoid the formation of bubbles in the wells. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.

1. Break off the required number of wells needed (number of samples plus 2 for controls) and place in holder. Return any unused wells to foil pouch with desiccant. Reseal pouch tightly to exclude moisture.
2. Using a micropipette, add **100 µl** of negative control to well # 1.
3. Using a micropipette, add **100 µl** of positive control to well # 2.
4. Add **100 µl** of diluted sample to the appropriate test wells.

NOTE: Place the opening of the transfer pipette just inside the well to avoid splashing into adjacent wells.

5. Cover the plate with parafilm or an appropriate cover and incubate for **60 minutes** at room temperature (15-25°C).
6. Decant the contents of the assay wells.
7. Wash each well using the 1X wash solution in a squirt bottle with a fine-tipped nozzle, directing the wash solution to the bottom of the well with force. Fill the well, and then decant the wash solution out of the well. Repeat this step four more times for a total of five washes.
8. After the last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.

NOTE: If using semi-automated or automated washing equipment, the specimens must be centrifuged (2000 to 3000g x 10 minutes) to remove any particulate matter prior to adding to the plate. Add **350 µl** of 1X wash solution to each well. Wash for a total of five times.

9. Add **100 µl (or 2 drops)** of Enzyme Conjugate to each well.
10. Cover the plate and incubate for **30 minutes** at room temperature (15-25°C).
11. Decant the contents of the wells and wash each well five times as in **Steps 6-8*.**
12. Add **100 µl (or 2 drops)** of Substrate Solution to each well.
13. Cover the plate and incubate for **10 minutes** at room temperature (15-25 ºC).
14. Add **100 µl (or 2 drops)** of Stop Solution to each well. Gently tap the wells to mix for approximately **15 seconds**. Read reaction within **5 minutes** after adding stop solution.
15. Read results visually or with a spectrophotometer having a dual wavelength of 450/620-650 nm. Zero reader on air.

Washings consist of vigorously filling each well to overflowing and decanting contents, banging the wells on a clean absorbent towel after each wash.

X. CALCULATION OF RESULTS

Interpretation of Results – Visual Method

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 - Spectrophotometric Method

Zero spectrophotometer against air. ***Read all wells at 450/620-650 nm***.

Positive: Absorbance reading of 0.08 O.D. units and above indicates the sample contains *Giardia* antigen.

Negative: Absorbance reading less than 0.08 O.D. units indicates the sample does not contain detectable levels of *Giardia* antigen.

XI. QUALITY CONTROL

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

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

XII. 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*.

XIII. PERFORMANCE CHARACTERISTICS

Clinical Evaluation

A total of 81 stool specimens were tested on the Biomerica *Giardia* ELISA and a reference ELISA for comparison. The clinical profile for the Biomerica *Giardia* ELISA is shown below.

		REFERENCE GIARDIA ELISA		
		POS	NEG	TOTAL
BIOMERICA GIARDIA ELISA	POS	11	5	16
	NEG	0	65	65
	TOTAL	11	70	81

Accuracy	94%
Sensitivity	100%
Specificity	93%

In a second study 90 stool specimens were tested on the Biomerica *Giardia* ELISA. 50 of the 90 samples tested were confirmed positive by Microscopy. 40 of the 90 samples tested were confirmed negative by Microscopy. The clinical profile for the Biomerica *Giardia* ELISA is shown below.

		MICROSCOPY		
		POS	NEG	TOTAL
BIOMERICA GIARDIA ELISA	POS	48	0	48
	NEG	2	40	42
	TOTAL	50	40	90

Accuracy	98%
Sensitivity	96%
Specificity	100%

Precision and Reproducibility

The precision (Intra-assay variation) of the Biomerica *Giardia* ELISA test was calculated from 24 replicate determinations on each of the two control samples.

Intra-Assay

Sample	Mean O.D	N	% CV
Control 1	1.86	24	5.2%
Control 2	0.89	24	5.1%

The reproducibility (Inter-assay variation) of the Biomerica *Giardia* ELISA test was calculated from data on two control samples obtained in 11 different assays by three technicians on two different lots of reagents.

Inter-Assay

Sample	Mean O.D	N	% CV
Control 1	1.5	11	7.8%
Control 2	0.7	11	9.8 %

Cross-Reactivity

The following bacterial strains at a concentration greater than 10^6 cell/mL were spiked into a confirmed negative fecal specimen. No cross-reactivity to the below bacterial strains was observed in the Biomerica *Giardia* ELISA.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Negative Sample - No Spike	N/A	0.002
Campylobacter coli 1114 - Spike	N/A	0.002
Campylobacter fetus - Spike	19438	0.006
Escherichia coli serotype 055:k59 (B5) - Spike	12014	0.003
Escherichia coli serotype 0111:K58 (B4) - Spike	33780	0.003
Escherichia coli serotype 0111:NM - Spike	43887	0.002
Escherichia coli serotype 0157.H7 - Spike	43890	0.003
Escherichia coli serotype 0124.NM - Spike	43893	0.003
Campylobacter jejuni - Spike	29428	0.002
Salmonella typhimurium - Spike	SA972229	0.002
Shigella sonneii - Spike	25931	0.004

Interference

The following bacterial strains at a concentration greater than 10^6 cell/mL were spiked into a confirmed positive fecal specimen. No interference to the below bacterial strains was observed in the Biomerica *Giardia* ELISA, as all positive samples remained positive.

Bacterial Strain	ATCC #	OD_{450nm}-OD_{630nm}
Positive Sample - No Spike	N/A	2.269
Campylobacter coli 1114 - Spike	N/A	1.411
Campylobacter fetus - Spike	19438	1.632
Escherichia coli serotype 055:k59 (B5) - Spike	12014	1.744
Escherichia coli serotype 0111:K58 (B4) - Spike	33780	2.003
Escherichia coli serotype 0111:NM - Spike	43887	1.856
Escherichia coli serotype 0157.H7 - Spike	43890	1.620
Escherichia coli serotype 0124.NM - Spike	43893	1.740
Campylobacter jejuni - Spike	29428	1.858
Salmonella typhimurium - Spike	SA972229	1.923
Shigella sonneii - Spike	25931	1.625

XIV. REFERENCES

1. Erlandsen, L.S., Meyer, E.A. 1984. *Giardia* and giardiasis: biology, pathogenesis and epidemiology. Plenum Press, New York.
2. Black, R. et. al.: Giardiasis in Day-Care Center: Evidence of Person to Person Transmission. *Pediatric*, #60 (4), October 1977, pp. 486-491.
3. Wolfe, M.S. 1979. Managing the patient with giardiasis: clinical, diagnostic and therapeutic aspects. Waterborne Transmission of Giardiasis. U.S. EPA. Pp. 39-52.
4. Stevens, D.P., and Mahmoud, A.A. 1980. Giardiasis: the rediscovery ofan ancient pathogen. Curr. Clin. Top. In Infect. Dis. 1: 195-207.
5. Intestinal Protozoa: Cinderellas of Parasitology. *ASM News*, Vol. 52 (10), 1986. pp. 521-522.
6. Craun, G.: Waterborne Giardiasis in the United States. *Lancet*, August 30, 1986, pp. 513-514.
7. 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.
8. Phillips, S.C., Mildvan, D., and Williams, D.C. 1981. Sexual transmission of enteric protozoa and helminthes in a venereal disease clinic population. New Eng. J. Med. 305: 603-606.
9. Kappus, K. and Juranek, D.: *Giardia* in the Well. *JAMA*, March 25, 1988, Vol. 259 (12), pp. 1810.
10. Smith, J.: Identification of Fecal Parasites in the Special Parasitology Survey of the College of American Pathologist. ALCP, Vol. 72 (2) August 1979. pp. 371-373.
11. Allison, M.C. et. al.: A Microscopic and Immunodiagnostic Search for Giardiasis in Patients with Gastrointestinal Disorders. *Scan J Gastroenterol*, 1988 #23, pp. 209-212.
12. Nash, T., Herrington, D. and Levine, M.: Usefulness of an Enzyme Linked Immunosorbent Assay for Detection of *Giardia* Antigen in Feces. *J Clin Micro*, July 1987, Vol. 25 (7), pp. 1169-1171.
13. 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.
14. Ungar, B. et. al: Enzyme Linked Immunosorbent Assay for the Detection of *Giardia Lamblia* in Fecal Specimens. *J Infect Dis*., January 1984, Vol. 149 (1), pp. 90-97.

XV. SYMBOLS

	Storage Temperature
	Lot Code
	Expiration
	Manufacturer
	Authorized Representative
	Caution, see instructions
	For in vitro diagnostic use
	Catalog No.

XVI. ORDERING INFORMATION

Contact: 
BIOMERICA, INC.
17571 Von Karman Avenue
Irvine, CA 92614 USA

Toll Free (800) 854-3002 Within the U.S.
Phone: (949) 645-2111

FAX: (949) 553-1231
E-Mail: bmra@biomerica.com

67069-01_eng.doc March 2012

according to IVDD 98/79/ EC
MDSS GmbH
Schiffgraben 41
D-30175 Hannover, Germany