MCC

EC REP CEpartner4U, 3951 DB; 13. NL. tel: +31 (0)6.516.536.26)

Medical Chemical Corp., 19430 Van Ness Avenue, Torrance, California 90501 • (310)787-6800, Fax (310)787-4464

PARA-TECT™
Giardia lamblia Antigen Detection Microwell ELISA Directions For Use For In Vitro Diagnostic Use

Catalog # MCC-GA-96, 96 Test

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.[1] 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]

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

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]

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 washing 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 2 mL of a diluted *Giardia* positive formalinized stool supernatant.
Negative control: One (1) vial containing 2 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. 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. Do not use this kit past the expiration date.

Storage Conditions

Reagents, strips and bottled components

Store between 2 - 8º C.

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

Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate (20X) to a squeeze bottle containing 475 mL of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings. Working wash buffer may be stored at room temperature (15-25º C) for up to one (1) year.

Specimen Collection and Preparation

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, Total-Fix or SAF. 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 –15 to –25º C or lower until used. Freezing does not adversely affect the test. Formalized, Total-Fix and SAF 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 diluted wash buffer.

Preparation of 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.

Processing of Preserved Stools (Formalin, Total-Fix and SAF)

Mix contents thoroughly inside collection container. No further processing is required.

Materials For Procedure

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. 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 µL) 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. Mix wells by tapping plate gently for 30 seconds.
4. Incubate for 30 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.*

5. Add 2 drops of Reagent 1 (blue solution) to each well.

6. Incubate for 5 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.*

7. Add 2 drops of Reagent 2 (red solution) to each well.

8. Incubate for 5 minutes at room temperature (15-25° C). Shake out contents of wells into discard container, then wash.*

9. Add 2 drops of Chromogen to each well.

10. Incubate 5 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.

12. Read results visually or at 450/620-650 nm within 60 minutes after adding Stop Solution. Zero reader on air.

* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times. After the third fill, decant the contents into a designated discard container, turn the plate upside down and slap dry against paper towel covered solid surface. Controls must be included each time the kit is run.

Interpretation of Results

Interpretation of Results - Visual (Manual

Reactive: Any sample well that is obviously more yellow than the negative control well.

Non-reactive: Any sample well that does not have obvious and significant yellow color.

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.*

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*.

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 MCC ELISA test and another manufacturer's ELISA kit.

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

% Positive Agreement = 91.7% (11/12)

% Negative Agreement = 95.9% (70/73)

Study #4

A total of 90 SAF preserved stools (44 positive and 46 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 +	39	0
ELISA -	5	46

Sensitivity: 88.6% (39/44)

Specificity: 100% (46/46)

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 fragili*s, *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 bacteria 1 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).

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. Call technical support for further instructions.

Troubleshooting

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

References

1. Black, R., A.C. Dykes, S.P. Sinclair, and J.G. Wells. *Giardiasis in day-care centers: evidence of person to person transmission*. Pediatrics. 1977. 60(4): 486-491.

2. Craun, G.F. *Waterborne giardiasis in the United States 1965-84*. Lancet. 1986. 2(8508): 513-514.

3. Smith, J. *Identification of fecal parasites in the Special Parasitology Survey of the College of American Pathologists*. Am. J. Clin. Path. 1979. 72(2): 371-373.

4. Kappus, K. and D. Juranek. *Giardia* in the Well. *JAMA*. 1988. 259(12): 1810.

5. Allison, M.C., E.L. Green, D.N. Bhattacharya, A. Smith, and R.E. Pounder. *A microscopic and immunodiagnostic search for giardiasis in patients with gastrointestinal disorders*. Scand. J. Gastroenterol. 1988. 23: 209-212.

6. Nash, T., D. Herrington, and M. Levine. *Usefulness of an enzyme-linked immunosorbent assay for detection of Giardia antigen in feces*. J. Clin. Microbiol. 1987. 25(7): 1169-1171.

7. Green, E., M. Miles, and D. Warhurst. *Immunodiagnostic detection of Giardia antigen in faeces by a rapid visual enzyme-linked immunosorbent assay*. Lancet. 1985. 2(8457): 691-693.

8. Peters, C.S., R. Sable, W.M. Janda, A.L. Chittom, and F.E. Kocka. *Prevalence of enteric parasites in homosexual patients attending an outpatient clinic.* J. Clin. Microbiol. 1986. 24(4): 684-685.

9. *Intestinal Protozoa: Cinderellas of Parasitology.* ASM News. Vol. 52 (10), 1986. pp. 521-522.

10. Burke, J. *Giardiasis in childhood*. Am. J. Dis. Child. 1975. 129(11): 1304-1310.

11. Danciger, M. and M. Lopez. *Numbers of Giardia in the feces of infected children*. Am. J. Trop. Med. Hyg. 1975. 24 (2): 237-242.

12. Ungar, B.L., R.H. Yolken, T.E. Nash, and T.C. Quinn. *Enzyme linked immunosorbent assay for the detection of Giardia lamblia in fecal specimens*. J. Infect. Dis. 1984. 149(1): 90-97.

Revised October 2010

Manufactured by:
Medical Chemical Corporation
19430 Van Ness Ave.
Torrance, CA 90501 USA
Telephone: 310-787-6800, FAX: 310-787-4464

www.med-chem.com