**MICRO.PARA.6.0 GIARDIA II WAMPOLE ELISA**

**PRINCIPLE**

The GIARDIA II test is an enzyme immunoassay for the qualitative detection of *Giardia lamblia* cyst antigen in human fecal specimens. It is indicated for use with fecal specimens from patients with diarrhea to determine the presence of *G. lamblia* gastrointestinal infection. This test can be used for fecal specimens submitted for routine clinical testing from adults or children.

*Giardia* is a binucleated flagellated protozoan parasite which exists in two forms: a noninfectious, pear-shaped trophozoite inhabiting the small intestine and the highly infectious cyst form which is elliptical in shape. The parasite is responsible for infections due to water contamination and travelers have been found to contract giardiasis from endemic areas. Transmission also occurs by direct contact especially by asymptomatic carries and by food contamination. High-risk categories include young children, immunocompromised patients, and those without previous exposure. More recently, giardiasis has become a common sexually transmitted disease.

The GIARDIA II test uses monoclonal and polyclonal antibodies to a cell-surface antigen of the organism. The Microassay Plate in the kit contains immobilized monoclonal antibody and the Conjugate consists of poly clonal antibody, both of which are specific for the cell-surface antigen. In the assay, an aliquot of diluted fecal specimen is transferred to a microassay well. The immobilized monoclonal antibody binds the Giardia antigen if the antigen is present. Upon addition, Conjugate then binds to the antigen/antibody complex. Any unbound materials are removed during the washing steps. Following the addition of the substrate, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of *Giardia* antigen and Conjugate.

**DOCUMENT OWNER**

Supervisor, Microbiology

**SPECIMEN**

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| **Specimen** | **Temperature** | **Stability** |
| Stool preserved with 10% buffered formalin  OR  Sodium Acetate Formalin (SAF) | Room temperature | 18 months |
| Unpreserved stool in sterile container | 2-8°C  Frozen | 24 hours |

If unpreserved specimen cannot be tested within 24 hours transfer to 10% buffered formalin.

Concentration steps are not recommended for fecal specimens. Make sure that specimens are thoroughly mixed (vortex) prior to performing the test.

**REAGENTS**

1. Conjugate, 7mL (rabbit polyclonal antibody to a cell-surface antigen of Giardia in a protein buffered solution containing 0.02% Thimerosal).
2. Diluent, 50mL (Buffered protein solution containing 0.02% Thimersal). The diluent is also to be used as the negative control.
3. Stop Solution, 7mL (0.6N sulfuric acid). **CAUTION**: Avoid contact with skin. Flush with water immediately if contact occurs.
4. Positive Control, 3.5mL (Giardia antigen in a protein buffered solution containing 0.02% thimerosal).
5. Substrate, 14mL (solution containing tetramethylbenzide and peroxide).
6. Wash Buffer Concentrate, 50mL (20x concentrate containing phosphate-buffered saline, detergent and 0.2% thimerosal).
7. Microassay Plate, 12 strips each consisting of 8 wells coated with monoclonal antibody to Giardia cell-surface antigen (stored with desiccant).

**EQUIPMENT**

1. Plastic adhesive sheets
2. Graduated disposable pipettes
3. Distilled water for dilution wash reagent
4. Squirt bottle for wash reagent
5. Absorbent paper
6. Applicator sticks
7. Vortex mixer
8. Discard container
9. Test tubes (12x75mm)
10. ELISA reader sample of reading 450nm or 450/620nm (Multiskan FC)
11. Timer
12. Disposable gloves

**QUALITY CONTROL**

1. A positive and a negative control must be run with each series of test specimens.
2. Each positive control well should be an easily visible yellow color and should give an absorbance of 0.500 or higher. Any well that gives a positive reading without visible color should be repositioned, wiped on the underside of the well, and read again.
3. Negative controls should be colorless or they may have a faint yellow color, but they must have an absorbance value of <0.150 when read on a single wavelength (OD450nm) or <0.090 when read on duel wavelength (OD450/620nm).
4. Test results are not valid unless performance characteristics of the positive and negative controls are met. If these results are not observed, call Technical Services.

**PROCEDURE**

1. Reagent Preparation

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| Step | Action |
| 1. | Bring the entire kit, including microwell pouch, to room temperature (21-27°C) before use. Return to 2°-8°C immediately after use. |
| 2. | Prepare a decontamination vessel for discarding reagents and materials. |
| 3. | Do not allow microwells to dry out between steps. |
| 4. | Reproducibility in any EIA is largely dependent on the consistency and thoroughness with which the microwells are washed. Carefully follow the recommended wash procedure as outlined in the ELISA test procedure. An automated washer may be used. |
| 5. | Prepare 1X wash buffer as needed. Add the 20X wash buffer concentrate to 950mL of distilled water. The wash solution can be stored at 2°-8°C. |
| 7. | Use plastic adhesive sheets to cover assay during incubation steps. Cut to size, and then remove paper backing before use. |

1. Specimen Preparation

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| If | Then |
| Fresh/frozen fecal specimen | Frozen fecal specimens should be thawed. Add 400uL of diluent to a test tube (one per sample), then add 100uL (2nd graduation mark on pipette) of sample to tube and mix well. This is a 1:5 dilution. If the specimen cannot be pipetted, use an applicator stick to transfer approximately 0.1 gram of feces. This is about the size of a small pea (4mm in diameter). Perform final dilution in the microassay wells as directed in test procedure. |
| Preserved fecal specimen | Mix (vortex) contents of container thoroughly before transferring specimen. No further processing or dilution is necessary. Perform final dilution in microassay wells as directed in test procedure. |

1. Test Procedure
   1. Manual ELISA Method

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| Step | Action |
| 1. | After reagent pouch has reached room temperature (22-27°C), break off the required number of microwells (one well for each specimen, plus two wells for positive and negative control per batch). Place the microwells in the microwell strip holder and record the location of all wells. Unused microwells must be resealed in the pouch immediately. |
| 2. | Add 1 drop (50uL) of the positive control (black cap) to the positive control well and 2 drops (100uL, 2nd graduation mark on pipette) of the negative control, i.e. diluent to the negative control well. |
| 3. | Transfer 100uL of diluent to each test well of the microassay plate. Using plastic pipettes, transfer 1 drop (50uL, 1st graduation mark on pipette) of sample (preserved or diluted as in specimen preparation) to each test well already containing diluent and gently tap the wells to mix. Seal with plate sealer and incubate 1 hour at room temperature. |
| 4. | Shake out contents of assay wells into a discard pan. Wash each well using the 1X wash solution in squirt bottle with fine-tipped nozzle, directing the wash solution to the bottom of well with force. Fill the wells, shake the wash solution out of the well into a discard pan and slap the plate hard onto a dry paper towel. Repeat the washing step 3 times (for a total of 4 washes). If any fecal material remains in the wells wash the plate until it appears clean. |
| 5. | After washing, completely remove any residual liquid in the wells by striking the plate onto a dry paper towel until no liquid comes out. Dispose of paper towels and specimen containers properly. |
| 6. | Add 1 drop (50uL) of conjugate (red top) to each well and gently tap to mix. Seal with plastic adhesive sheet. Incubate wells for 30 minutes at room temperature. |
| 7. | Repeat washing procedure as described in steps 4 and 5. |
| 8. | Add 2 drops (100uL) of substrate (blue cap) to each well. Gently tap the wells to mix. Incubate wells at room temperature for 10 minutes. |
| 9. | Add 1 drop (50uL) of Stop solution (yellow cap) to each well. Gently tap the wells and wait 2 minutes before reading. |
| 10. | Observe reactions using spectrophotometric or visual determination. |

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| If reading by | Then |
| Multiskan FC Microplate Reader (Primary reader) | 1. Read within 30 minutes of adding Stop Solution. Zero EIA reader on air prior to reading specimens. 2. Click on SkanIt for Multiskan FC 3.1 icon to open software. Plate reader drawer will open to accept microplate for evaluating. Username is ‘admin.’ Leave password blank, 3. Select GIAIA from the Recent Sessions menu. Click on the Layout tab. 4. With left mouse button, click and hold the number of wells needed on the well screen. Right click on one of the highlighted wells and select ‘fill with unknowns.’ Click OK. 5. Place plate into reader and press start. 6. Change name for session to GIAIA\_mmddyyyy then press OK. 7. After reading, click on Reports tab and click print. Close template (select YES to save results). Close all windows. |
| Visual determination (should only be used if there are no other methods available) | 1. Read 2 minutes after addition of stop solution. 2. Examine wells for the presence of a yellow color. |

**INTERPRETATION OF RESULTS**

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| Method | Negative | Positive |
| Spectrophotometric determination (dual wavelength 450/620nm) | OD < 0.090 | OD ≥ 0.090 |
| Visual determination | Colorless to faint (barely visible) yellow | Definite yellow color |

**REPORTING RESULTS**

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| Result | Sunquest | QLS |
| Positive | a. Click on MICRO RESULT ENTRY in Misys Gateway.  b. Type the accession number in the VALUE box.  c. Click on the test desired and click on SELECT.  d. Use shortcut key ‘R’ or code ‘DGIA’  e. Final and save report.  f. Print an IRA to report the positive result to the Indiana State Department of Health.  g. If inpatient, telephone positive result to the unit or physician. | a. Function: 3,3,1  b. <Enter> to worklist prompt type CRGIA and <enter>.  c. <Enter> to accession prompt. Type the accession number and <enter>.  d. Type ‘D’ at GIAIT prompt <enter>.  e. At RELEASE ALL prompt type ‘Y’ and <enter>.  f. Print a patient report to report the positive result to the Indiana State Department of Health.  g. If inpatient, telephone positive result to the unit or physician. |
| Negative | a. Click on MICRO RESULT ENTRY  b. Type the accession number in the VALUE box.  c. Click on test desired and click on SELECT.  d. Use shortcut key ‘W’ or code ‘NDGIA.’  e. Final and save report. | a. Function: 3,3,1  b. <Enter> to worklist prompt type CRGIA and <enter>.  c. <Enter> to accession prompt. Type accession number and <enter>.  d. Type ‘N’ at GIAIT prompt <enter>.  e. At RELEASE ALL prompt type ‘Y’ and <enter>. |

**LIMITATIONS**

1. The GIARDIA II test detects the presence of *Giardia* antigen in fecal specimens. This antigen is present primarily in the cyst form of the parasite.
2. The test results should be interpreted by a physician in consideration of other laboratory results and clinical history.
3. Concentrated fecal specimens should not be tested in the GIARDIA II test and will not give accurate results.
4. The predictive value of a positive results decreases when testing in a low prevalence population.
5. The GIARDIA II test is for the qualitative detection of *Giardia* antigen in fecal specimens. It has not been evaluated for quantitative determinations of organism load, and the magnitude of the absorbance value does no correlate with organism load.

**REFERENCES**

Wampole GIARDIA II package insert. TECHLAB. Blacksburg, Virginia. Issued 10/2006.