**MICRO.PARA.7.0 CRYPTOSPORIDIUM II WAMPOLE ELISA**

**PRINCIPLE**

*Cryptosporidium* spp. Is a protozoan parasite of vertebrates previous thought to cause diarrhea only in animals. In 1976, the first human infection was reported. Since then it has been found to be associated with diarrheal illness in most parts of the world and is a frequent cause of travelers’ diarrhea.  
  
The disease is transmitted by the thick-walled oocyst form, 2-6um in diameter, which is remarkably resistant to common disinfectants and routine chlorination of drinking water. Person-to-person transmission, especially among children is common. *Cryptosporidium* has little or no host specificity, and animals such as rodents, cattle, and domestic pets serve as a reservoir for zoonotic transmission to humans. Such transmission occurs either by direct contact or by contamination of water supplies with fecal matter. Cryptosporidiosis is a serious opportunistic infection in patient with acquired immunodeficiency syndrome (AIDS) and is potentially a sexually transmitted disease. Clinical manifestations of cryptosporidiosis include cholera-like diarrhea, abdominal pain, nausea, and vomiting and weight loss. In normal persons the infection is usually self-limiting and of short-term. In AIDS and other immunosuppressed patients, cryptosporidiosis can result in prolonger and self-threatening illness due to excessive fluid loss. In these patients, the infection may also spread to the respiratory and biliary tracts.  
  
The CRYPTOSPORIDIUM II test uses monoclonal and polyclonal antibodies to *Cryptosporidium* oocyst antigen. The Microassay Plate in the kit contains an immobilized monoclonal antibody against *Cryptosporidium* oocyst antigen, and the Conjugate consists of a polyclonal antibody against *Cryptosporidium* oocyst antigen. In the assay, an aliquot of a diluted fecal specimen is transferred to a microassay well. If *Cryptosporidium* oocyst antigen is present, it binds to the immobilized monoclonal antibody. Upon addition, Conjugate then binds to antigen/antibody complex. Any unbound materials are moved during the washing steps. Following the addition of substrate, a color is detected due to the enzyme-antibody-antigen complexes that formed in the presence of *Cryptosporidium* oocyst 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)  OR  Transport media (Cary Blair and C&S) | 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 Cryptosporidium oocyst antigen coupled to a horseradish peroxidase 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 (Heat-inactivated bovine fecal material containing Cryptosporidium oocyst 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 Cryptosporidium oocyst 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 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 0.1gram 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. | Shake the Cryptosporidium positive control bottle (white cap) for several seconds. Then add 1 drop (50uL) 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 4 times (for a total of 5 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 CRYIA1 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 CRYIA1\_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 Accession number in the VALUE box.  c. Click on test desired and click on SELECT.  d. Use shortcut key ‘]’ or code ‘DCRY’  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 accession number and <enter>  d. Type ‘D’ at CRYIT 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 Accession number in the VALUE box.  c. Click on test desired and click on SELECT.  d. Use shortcut key ‘[’ or code ‘NDCRY’  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 CRYIT prompt <enter>.  e. At RELEASE ALL prompt type ‘Y’ and <enter>. |

**LIMITATIONS**

1. The CRYPTOSPORIDIUM II test detects the presence of *Cryptosporidium* oocyst antigen in fecal specimens.
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 CRYPTOSPORIDIUM II test and will not give accurate results.
4. The CRYPTOSPORIDIUM II test is intended for the qualitative detection of *Cryptosporidium* oocyst 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 CRYPTOSPORIDIUM II package insert. TECHLAB. Blacksburg, Virginia. Issued 08/2006.