



PARA-TECT™
Cryptosporidium/Giardia
Direct Fluorescent Assay
Directions For Use
For In Vitro Diagnostic Use

Catalog # MCC-C/G - DFA, 75 Test

Intended Use

This direct fluorescent assay (DFA) is an in vitro immunoassay for the qualitative determination of *Cryptosporidium* and *Giardia* oocysts and cysts in feces. This assay may be used with stools preserved in 10% formalin, SAF or MCC's Universal Fixative.

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.³

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.¹⁶

Cryptosporidium is a coccidian parasite that is recognized as an important enteric pathogen. The organism causes an acute, though self-limiting infection in immunocompetent individuals. Incubation periods of 1 to 12 days have been reported with most oocyst shedding ending by day 21. Symptoms range from mild to severe diarrhea with a variety of complications.^{5,6,7,8,9,10}

The infection in immunocompromised patients is much more severe and may often be life threatening. Passage of fluid, up to 12 liters per day, has been reported.^{5,11,12,13,14,15}

Multiple pathways of *Cryptosporidium* transmission have been implicated. These include animal to human, water contamination and person-to-person. The latter may include contact between members of the same household, day care centers, and homosexual men.^{5,11,13,14,15}

Principle of Procedure

This assay utilizes the principle of direct immunofluorescence. The conjugate contains a mixture of FITC-labeled monoclonal antibodies directed against *Cryptosporidium* oocysts and *Giardia* cysts, which, if present, are affixed to the slide. The slide is then rinsed to remove unbound conjugate and examined under a fluorescent microscope looking for an apple-green color and the characteristic morphology of the *Cryptosporidium* oocysts and the *Giardia* cysts.

Reagents

Conjugate: One 4 ml vial of FITC-labeled anti-*Cryptosporidium* and anti-*Giardia* monoclonal antibodies

Positive Control: One 1 ml vial of formalinized stool supernatant containing *Cryptosporidium* oocysts and *Giardia* cysts

Negative Control: One 1 ml vial of formalinized stool supernatant

Wash Buffer: One 25 ml bottle of concentrated (20X) buffer

Counterstain: One 4 ml bottle of Eriochrome Black

Mounting Media: One 3 ml vial

Transfer loops: One package of 80

Slides: One package of 25, 3-well treated slides

Slide Coverslips: One Package of 25

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. Persons who are color blind or visually impaired may not be able to read the test and should use another method (such as an EIA) to test the samples. Incubation Stage 2 should be performed in a light protected area. Take care not to scratch the surface of the slide test wells.

Storage Conditions

Store between 2 - 8° C. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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 preserved in 10% formalin, SAF, MCC's Unifix or Total-Fix. Preserved samples may be kept at room temperature (15-25° C) or refrigerated.

Wash Buffer Preparation – Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water.. Swirl to mix.

Preparation of Preserved Stools

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

Samples with low numbers of oocysts or cysts may benefit by concentration of the sample prior to testing. Use of the Micro-Sed, Sed-Connect and Para-Sed concentration kits (Medical Chemical Corporation, Torrance, CA) have been validated with this assay.

Procedure

Materials Provided

Cryptosporidium/Giardia DFA Kit

Materials Required But Not Provided

Applicator Sticks

Squeeze bottle for washing strips

Graduated Cylinder

Reagent grade (DI) water

Humidity Chamber (place in light protected area)

Fluorescent Microscope (excitation wavelength of 490-500, barrier filter of 510-530)

Proper Temperature

All incubations are at room temperature (15 to 25° C)

Test Procedure

INITIAL SETUP

1. Bring the 20X Wash Solution to 500 ml with distilled water. **MIX WELL**
2. Fill a squeeze bottle with the resulting 1X wash buffer.
3. Thoroughly mix all reagents and specimens before use

INCUBATION STAGE 1

4. Use a new transfer loop to transfer the Positive Control to a slide well. One can also use a transfer pipet to dispense one drop onto the slide. Carefully spread the material over the entire well taking care not to scratch the treated surface.
5. Use a new transfer loop to transfer the Negative Control to a slide well. One can also use a transfer pipet to dispense one drop onto the slide. Carefully spread the material over the entire well taking care not to scratch the treated surface.
6. Use a new transfer loop to transfer each patient's stool specimen to a slide well. Carefully spread the material over the entire well

taking care not to scratch the treated surface.

7. Allow the slides to completely air-dry. This process usually takes between **20-40 minutes** depending upon environmental conditions.

INCUBATION STAGE 2

8. Add 50 µL (1 drop) of the Conjugate to all used slide wells

9. Add 50 µL (1 drop) of the Counterstain to all used slide wells

10. Mix reagents with applicator stick and spread over entire well taking care not to scratch the treated surface.

11. Incubate the slides in a **light-protected** humidity chamber for **30 minutes**.

RINSE STAGE

12. Using the squeeze bottle from step 2, gently rinse the slides to remove excess conjugate and counterstain.

13. Tap the edge of the slide against a paper towel to remove excess buffer. **DO NOT ALLOW SLIDE TO DRY.**

14. Add 1 drop of mounting medium to each well and apply coverslip.

INTERPRETATION STAGE

15. Scan each well at 400X looking for the presence of the characteristic FITC brilliant apple-green color and morphology identified in the Quality Control Section.

Results

Cryptosporidium Positive:

The presence of at least one oocyst with the characteristic FITC brilliant apple-green color and morphology identified in the Quality Control Section.

Giardia Positive:

The presence of at least one cyst with the characteristic FITC brilliant apple-green color and morphology identified in the Quality Control Section.

Negative:

The absence of the characteristic FITC brilliant apple-green color and morphology identified in the Quality Control Section.

Limitation of Procedure

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. The presence of *Cryptosporidium* oocysts and *Giardia* cysts does not preclude concurrent infections by other pathogens.

Expected Values

Normal healthy individuals should be free of *Cryptosporidium* and *Giardia* and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of oocysts or cysts. This shedding may continue in some patients even after clinical recovery. Shedding may also occur in asymptomatic cases.

Specific Performance Characteristics

Study #1

A total of 170 unconcentrated stools (145 human stools and 25 bovine stools) examined by O&P microscopy were tested against the IVD DFA kit. The following results were obtained.

	Micro +	Micro -
DFA +	46	0
DFA -	0	124

For Giardia:

Sensitivity: 100% 95% CI = 92% to 100%

Specificity: 100% 95% CI = 97% to 100%

	Micro +	Micro -
DFA +	39	0
DFA -	0	131

For *Cryptosporidium*:

Sensitivity: 100% 95% CI = 91% to 100%

Specificity: 100% 95% CI = 97% to 100%

Study #2

A total of 53 unconcentrated stools examined by O&P microscopy were tested against the IVD DFA kit. The following results were obtained.

	Micro +	Micro -
DFA +	16	0
DFA -	0	37

For *Giardia*:

Sensitivity: 100% 95% CI = 79% to 100%

Specificity: 100% 95% CI = 90% to 100%

	Micro +	Micro -
DFA +	11	0
DFA -	0	42

For *Cryptosporidium*:

Sensitivity: 100% 95% CI = 72% to 100%

Specificity: 100% 95% CI = 92% to 100%

Study #3

A total of 74 formalin and SAF preserved stools were tested against the IVD DFA test.

	Micro +	Micro -
DFA +	26	0
DFA -	0	48

For *Giardia*:

Sensitivity: 100% 95% CI = 87% to 100%

Specificity: 100% 95% CI = 93% to 100%

	Micro +	Micro -
DFA +	18	0
DFA -	1	55

For *Cryptosporidium*:

Sensitivity: 95% 95% CI = 74% to 100%

Specificity: 100% 95% CI = 94% to 100%

No cross-reactions were seen with the following organisms:

Entamoeba hartmanni, *Endolimax nana*, *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Blastocystis hominis*, *Dientamoeba fragilis*, *Chilomastix mesnili*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Diphyllobothrium* species, *Hymenol-epis nana*, *Enteromonas hominis*, *Trichuris trichiura*, *Iodamoeba buetschlii*, Hookworm, *Taenia* eggs, White Blood Cells, *Cyclospora cayetanensis*.

Summary of Reproducibility Data

Nine samples (3 negative, 3 *Giardia* positive and 3 *Cryptosporidium* positive) were tested by 3 different persons. All 3 people correctly identified the negative and positive samples.

Quality Control

The positive and negative controls must be included whenever patient testing performed.

Positive Control: Must show the presence of both the *Cryptosporidium* oocyst and the *Giardia* cyst. *Cryptosporidium* oocysts are 2-6µm in size are round to slightly ovoid in shape. The oocyst wall will stain the characteristic FITC brilliant apple-green color. *Giardia* cysts are 8-12µm in size and are oval shaped. The cyst wall will stain the characteristic FITC brilliant apple-green color.

Negative Control: Must not exhibit any of the characteristic FITC brilliant apple-green color associated with either the oocyst or cyst morphology

Background Staining: The background material in the control wells and patient wells will counterstain a dull orange-red color. There also may be non-specific fluorescence that is easily distinguished from the brilliant apple-green color and morphology of the oocyst and cyst.

References

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