

ColorPAC™ *Giardia*/*Cryptosporidium* Rapid Assay

For the Qualitative Detection of *Giardia* and *Cryptosporidium* Specific Antigens

I. INTENDED USE

The Becton Dickinson **ColorPAC™ *Giardia*/*Cryptosporidium*** test is a rapid immunoassay for the qualitative detection of *Giardia* and *Cryptosporidium* specific antigens in aqueous extracts of fecal specimens. It is intended for *in vitro* diagnostic use as an aid in the detection of suspected *Giardia* or *Cryptosporidium* infections by professional laboratories.

II. SUMMARY AND EXPLANATION

Giardia and *Cryptosporidium* are recognized as two of the most frequent causes of parasitic intestinal disease. Both organisms are found throughout the world. Transmission is usually through the ingestion of contaminated food or water.

Giardiasis in humans is caused by the protozoan parasite *Giardia lamblia* (also known as *Giardia intestinalis*). The acute disease is characterized by watery diarrhea, nausea, abdominal cramps, bloating, weight loss and malabsorption lasting for several weeks. Chronic or asymptomatic infection can also occur.^{1,2}

Cryptosporidiosis in humans is caused by the coccidian parasite *Cryptosporidium parvum*. Acute symptoms include watery diarrhea, abdominal cramps, loss of appetite, low grade fever, nausea and vomiting lasting for several days to over a month. Severe, persistent infections can occur in immunocompromised patients.¹ Infection may also be asymptomatic. The parasite has been implicated in several major waterborne outbreaks in the United States.³

Diagnosis of *Giardia* and *Cryptosporidium* infection has traditionally been done by microscopic examination of stools. More recently, the detection of *Giardia* and *Cryptosporidium* antigens in stool specimens using enzyme immunoassays has become an accepted approach to diagnosis.^{4,6} The **ColorPAC *Giardia*/*Cryptosporidium*** assay detects similar antigens using a non-enzymatic rapid immunoassay format.

III. PRINCIPLE OF THE PROCEDURE

The **ColorPAC *Giardia*/*Cryptosporidium*** test is a qualitative immunochromatographic assay that simultaneously detects and distinguishes between *Giardia* and *Cryptosporidium* antigens in aqueous extracts of patient stool specimens. The specimen, collected in a sample transport or preservative medium, is added to a tube containing a treatment buffer. A biotinylated anti-*Giardia* capture antibody reagent is then added, followed by a pooled suspension of colloidal dye labeled monoclonal antibodies to *Giardia* and *Cryptosporidium*. The sample is then mixed and poured into the test device that contains a capture reagent (an avidin derivative) for *Giardia*, a capture antibody for *Cryptosporidium*, and a control antibody that binds to excess colloidal dye conjugate. If *Giardia* antigen is present in the sample, a black band will develop at the GIAR. position in the device window. If *Cryptosporidium* antigen is present, a black band will appear at the CRYP. position.

The appearance of a black band at the CONT. position is required for the test result to be valid. It indicates that the colloidal dye conjugate is intact and that proper capillary flow has occurred.

IV. KIT COMPONENTS AND REAGENTS

Foil pouched **ColorPAC** *Giardia/Cryptosporidium* test devices consisting of: a) a membrane coated with an avidin derivative, mouse anti-*Cryptosporidium* and goat anti-mouse IgG, b) pad materials, desiccant and a plastic housing. (30 per test kit)

- (1.5 mL) Sample Treatment Buffer: Buffer solution with detergent, contains azide 0.1%.
- (1.2 mL) Conjugate Reagent A: Biotinylated rabbit anti-*Giardia* in diluent buffer with carrier protein and detergent, contains azide 0.1%.
- (1.2 mL) Conjugate Reagent B: Colloidal dye labeled monoclonal antibodies to *Giardia* and *Cryptosporidium* in diluent buffer with carrier protein and detergent, contains azide 0.1%.
- (1 bag of 30) Specimen transfer pipets.
- (1 bag of 30) Specimen dilution tubes.

Procedure Chart

Product Information

Precautions: For In Vitro Diagnostic Use.

After review by the Centers for Disease Control and Prevention (CDC), under CLIA '88, this product has been identified as Moderate complexity. The CDC Analyte Identifier Code for *Giardia lamblia* is 2222 and for *Cryptosporidium* is 1109; the CDC Test System Identifier Code is 08172.

Do not use kit beyond the printed expiration date. Do not interchange or mix components from different kit lots.

Warning: Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The Food and Drug Administration recommends such material be handled at a Biosafety Level 2. BSL 2 is referenced in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual, Biosafety in Microbiological and Biomedical Laboratories.

Warning: Reagents contain sodium azide. Very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Wear eye/face protection. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Storage: Refrigerate at 2° – 8°C. DO NOT FREEZE.

V. SPECIMEN COLLECTION AND HANDLING

Stool specimens collected for ova and parasite examination can be used in the **ColorPAC Giardia/Cryptosporidium** assay. The samples should be collected in clean, leak-proof plastic containers.

Specimens in SAF, 10% formalin, MIF, Cary-Blair and Stuart’s transport media are acceptable. Samples in PVA are not suitable.

| | | |
|---|---|--|
| Formulin and SAF preserved specimens can be stored frozen (-20°C or -70°C), refrigerated (2°C - 8°C) or at room temperature (20°C - 30°C) and should be tested within 2 months after collection | Cary Blair diluted samples can be stored refrigerated (2°C- 8°C) and tested within 2 weeks after collection or frozen (-20°C) and tested within 2 months. | MIF preserved specimens can be stored frozen (-20°C), refrigerated (2°C- 8°C) or at room temperature (2°C - 8°C) and should be tested within 2 months after collection |
|---|---|--|

Specimens in Stuart’s medium should be tested as soon as possible after collection, since storage in this medium has not been validated.

Materials Provided: All materials as listed under “Kit Components and Reagents”.

Materials Required But Not Provided: Specimen collection and transport devices. SAF, 10% formalin, MIF, Cary-Blair and Stuart’s transport media are acceptable, clock or timer.

VI. PROCEDURES

1. Allow kit components and specimens to equilibrate to room temperature before use. Mix liquid reagents by inverting several times before use.
2. Do not remove the test devices from the pouch until ready for use.
3. Several tests may be run at the same time. Use separate dilution tubes and pipets for each specimen.
4. To prevent possible contamination, avoid touching the dispensing tip of the Sample Treatment Buffer, Conjugate Reagent A and Conjugate Reagent B dropper bottles to the dilution tubes, pipets, test devices, or anything that has come into direct contact with patient specimens.
5. It is important to dispense full drops of reagents for optimal assay performance. For best results, hold the bottle inverted in a near vertical position, squeeze slowly and allow air to enter the bottle between samples. Shaking the bottle slightly in an inverted position may help to dislodge air trapped in the dispensing tip.
6. Do not concentrate patient specimens. Specimens should be mixed to ensure uniform sampling, although it is acceptable to allow large particulates to settle so that the sample can be more easily pipetted.
7. Undiluted specimens should be diluted approximately 1:4 in water or one of the acceptable transport media to be run in the assay.

Test Procedure

1. Remove the test device from the pouch and place on a flat surface. Place a Specimen Dilution Tube into the kit workstation holder or into a suitable test tube rack.
2. Add two drops of Sample Treatment Buffer to the Specimen Dilution Tube.
4. Use the Specimen Transfer Pipet to aspirate the aqueous patient stool specimen by squeezing the upper bulb, inserting the open end into the sample and releasing pressure on the bulb while holding the pipet. Be sure to fill the barrel of the pipet completely. An overflow chamber prevents over-filling of the pipet. Transfer the contents of the pipet (approximately 60 µl) into the Specimen Dilution Tube.
4. Add two drops of Conjugate Reagent A to the tube.
5. Add two drops of Conjugate Reagent B to the tube.
6. Mix the sample by manual swirling or by vortexing. Pour the entire contents of the tube into the sample well of the test device.
4. Visually read the test results after 10 min. Results are invalid after 15 minutes.

VII. INTERPRETATION OF RESULTS

Positive for *Giardia*

The presence of grey-black bands at the GIAR. and the CONT. positions indicates that *Giardia* antigen has been detected. The intensity of the band can vary from faint to strong. Visible test lines of any intensity should be read as positive.

Positive for *Cryptosporidium*

The presence of grey-black bands at the CRYP. and the CONT. positions indicates that *Cryptosporidium* antigen has been detected. The intensity of the band can vary from faint to strong. Visible test lines of any intensity should be read as positive.

Negative for *Giardia*

No band present at the GIAR. position and a band is present at the CONT. position indicates that *Giardia* antigen is absent or is below detectable levels. The CRYP. position can have either a band or no band.

Negative for *Cryptosporidium*

No band present at the CRYP. position and a band is present at the CONT. position indicates that *Cryptosporidium* antigen is absent or is below detectable levels. The GIAR. position can have either a band or no band.

Invalid Results

No band appears at the CONT. position. Incomplete or beaded band appears at the CRYP or GIAR position. The test should be repeated using another device. In situations where adequate flow does not occur due to excessive particulate matter in the specimen, the sample can be diluted two-fold in water or in the same transport medium as it was originally collected and re-run.

VIII. QUALITY CONTROL

Several features are incorporated into the **ColorPAC** *Giardia/Cryptosporidium* test as routine quality checks:

1. The appearance of a control band at the CONT. position verifies that a functionally intact colloidal dye conjugate has been added to the device, that the control line antibody is functionally active and that adequate capillary flow has occurred.
2. The sequence of reagent additions to the sample tube allows the user to visually monitor the procedure: Sample Treatment Buffer is added to an empty tube; the stool specimen is visually observable; Conjugate Reagent A has a red dye incorporated in it; and Conjugate Reagent B is black in color.
3. Characterized formalin preserved patient specimens may be used as routine external positive and negative controls. Characterized patient samples should be run as test specimens according to the procedures described below.
4. It is recommended that characterized specimens be run as controls with each new kit lot or with each new operator.

Do not use the kit if controls have not yielded the appropriate results

IX. LIMITATIONS OF THE PROCEDURE

1. As with all diagnostic procedures, the results obtained with the **ColorPAC** *Giardia/Cryptosporidium* test should be used in conjunction with other clinical information available to the physician.
2. Negative results can occur in samples containing levels of antigen below the lower limits of detection of the assay. Multiple specimens collected over several days can be tested for patients suspected of being positive for *Giardia* or *Cryptosporidium*.

X. EXPECTED VALUES

The prevalence of *Giardia* and *Cryptosporidium* is variable among different populations and geographic areas. *Giardia* incidence in developed countries is approximately 2-5%; *Cryptosporidium* incidence in Europe and North America is about 1-3%.³ Higher prevalence rates may be present in children and in the immunosuppressed.¹⁻³

XI. PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

A multi-site clinical evaluation of the Becton Dickinson ColorPAC *Giardia/Cryptosporidium* test was performed both prospectively and retrospectively. Results from the ColorPAC *Giardia/Cryptosporidium* assay were compared with microscopic ova and parasite examination.

In the retrospective study, a panel of previously-characterized formalin preserved patient specimens was used. The **ColorPAC** assay was compared with microscopic examination results. Microscopic examination included standard ova and parasite staining for *Giardia* and other parasites, modified acid-fast staining for *Cryptosporidium* and if discrepancies were encountered, specific immunofluorescent staining for *Giardia* and *Cryptosporidium*.

Giardia results:

| | | ColorPAC™ <i>Giardia</i> result | |
|-------------------------------------|---|--|-----|
| | | + | - |
| Microscopic exam for <i>Giardia</i> | + | 33 | 0 |
| | - | 0 | 109 |

Sensitivity: $33/33 = 100\%$ (95% confidence interval 89.4 – 100%)

Specificity: $109/109 = 100\%$ (95% confidence interval 96.7 – 100%)

| | | ColorPAC™ <i>Giardia</i> result | |
|------------------------------|---|--|-----|
| | | + | - |
| Rapid EIA for <i>Giardia</i> | + | 29 | 0 |
| | - | 4 | 109 |

The four discrepant specimens were positive for *Giardia* by microscopy.

Relative* agreement between the **ColorPAC** assay and rapid *Giardia* EIA: $138/142 = 97.2\%$.

*Note: Please be advised the "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

Cryptosporidium results:

| | | ColorPAC™ Cryptosporidium result | |
|---|---|---|-----|
| | | + | - |
| Microscopic exam for <i>Cryptosporidium</i> | + | 36 | 1 |
| | - | 0 | 105 |

Sensitivity: $36/37 = 97.3\%$ (95% confidence interval 85.8-100%)

Specificity: $105/105 = 100\%$ (95% confidence interval 96.6-100%)

The one discrepant was verified as *Cryptosporidium* positive by immunofluorescence and probably contained a level of antigen below the limit of detection of the **ColorPAC** assay.

| | | ColorPAC™ Cryptosporidium result | |
|--------------------------------------|---|---|-----|
| | | + | - |
| Rapid EIA for <i>Cryptosporidium</i> | + | 36 | 0 |
| | - | 0 | 106 |

Relative* agreement between the **ColorPAC** assay and rapid *Cryptosporidium* EIA: $142/142 = 100\%$

*Note: Please be advised the "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

The prospective study was conducted on specimens characterized at three different geographically separated sites in the United States. A total of 502 specimens were tested using the **ColorPAC Giardia/Cryptosporidium** rapid test. Results were compared with microscopic examinations for ova and parasites combined with modified acid fast staining for *Cryptosporidium*.

Giardia results:

| | | ColorPAC™ Giardia result | |
|-------------------------------------|---|---------------------------------|-----|
| | | + | - |
| Microscopic exam for <i>Giardia</i> | + | 50 | 0 |
| | - | 4 | 448 |

Sensitivity: $50/50 = 100\%$ (95% confidence interval 92.9 – 100%)

Specificity: $448/452 = 99.1\%$ (95% confidence interval 97.8 – 99.8%)

Four putative false positives were encountered with the **ColorPAC** test. Discrepant testing showed all four samples to be from patients who were *Giardia* positive by microscopy on a different specimen.

Cryptosporidium results:

| | | ColorPAC™ <i>Cryptosporidium</i> result | |
|---|---|--|-----|
| | | + | - |
| Microscopic exam for <i>Cryptosporidium</i> | + | 73 | 0 |
| | - | 2 | 427 |

Sensitivity: $73/73 = 100\%$ (95% confidence interval 95.1 – 100%)

Specificity: $427/429 = 99.5\%$ (95% confidence interval 98.3 – 99.9%)

There were two putative *Cryptosporidium* false positives from the above data. These samples came from two patients for which *Cryptosporidium* could not be documented by a repeat of the microscopic examinations.

Assay Reproducibility

A panel of fifteen samples consisting of five replicates of a *Giardia* low level positive sample, five replicates of a *Cryptosporidium* low level positive sample and five replicates of a negative sample were run on five separate occasions by personnel at two diagnostic parasitology laboratories. Intra-assay reproducibility was demonstrated by 100% agreement (0% CV) among all of the replicate samples within each run. Inter-assay reproducibility was demonstrated by 100% agreement in the results among all of the ten separate occasions that the test panel was run.

Cross-reactivity

The **ColorPAC™** *Giardia/Cryptosporidium* assay was run on stool specimens documented to be positive for other parasites by microscopy.

The *Giardia*-specific part of the assay showed no cross-reactivity to the following organisms:

| | | |
|-------------------------------------|--|--------------------------------------|
| <i>Ascaris lumbricoides</i> (1) | <i>Entamoeba coli</i> (14) | <i>Iodamoeba bütschlii</i> (13) |
| <i>Blastocystis hominis</i> (58) | <i>Entamoeba hartmanni</i> (12) | Microsporidia (1) |
| <i>Chilomastix mesnili</i> (5) | <i>Entamoeba histolytica/dispar</i> (14) | <i>Strongloides sterocoralis</i> (2) |
| <i>Cryptosporidium parvum</i> (121) | <i>Enterobius vermicularis</i> (1) | <i>Taenia</i> sp. (1) |
| <i>Cyclospora cayetanensis</i> (1) | <i>Enteromonas hominis</i> (2) | <i>Trichomonas hominis</i> (1) |
| <i>Dientamoeba fragilis</i> (14) | Hookworm (1) | |
| <i>Endolimax nana</i> (28) | <i>Hymenolepis nana</i> (3) | |

The numbers in parentheses represent the number of samples tested for each organism.

The *Cryptosporidium*-specific part of the assay showed no cross-reactivity to the following organisms:

| | | |
|------------------------------------|--|--------------------------------------|
| <i>Ascaris lumbricoides</i> (1) | <i>Entamoeba coli</i> (21) | <i>Hymenolepis nana</i> (4) |
| <i>Blastocystis hominis</i> (92) | <i>Entamoeba hartmanni</i> (20) | <i>Iodamoeba bütschlii</i> (12) |
| <i>Chilomastix mesnili</i> (6) | <i>Entamoeba histolytica/dispar</i> (19) | Microsporidia (1) |
| <i>Cyclospora cayetanensis</i> (1) | <i>Enteromonas hominis</i> (2) | <i>Strongyloides stercoralis</i> (2) |
| <i>Dientamoeba fragilis</i> (16) | <i>Giardia lamblia</i> (132) | <i>Taemia sp.</i> (2) |
| <i>Endolimax nana</i> (48) | Hookworm (1) | <i>Trichomonas hominis</i> (1) |

The numbers in parentheses represent the number of samples tested for each organism.

XII. REFERENCES

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4. Rosoff, J.D., C.A. Sanders, S.S. Sonnad, P.R. De Lay, W.K. Hadley, F.F. Vincenzi, D.M. Yajko and P.D. O'Hanley, "Stool diagnosis of giardiasis using a commercially available enzyme immunoassay to detect Giardia-specific antigen 65 (GSA 65)", J. Clin. Microbiol. 27: 1997-2002. 1989.
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5. Garcia, L.S. and R.Y. Shimizu, "Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of Giardia lamblia and Cryptosporidium parvum in human fecal specimens," J. Clin. Microbiol. 35: 1526-1529. 1997.

XIII. AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 240909 | ColorPAC™ <i>Giardia/Cryptosporidium</i> Rapid Assay test, 30 test. |

XIV. TECHNICAL INFORMATION:

In the United States, telephone Becton Dickinson Microbiology Systems Technical Services toll free (800) 638-8663, Prompt2.

Approved By: _____

Date Effective: _____

Supervisor: _____ Date: _____

Director: _____ Date: _____

Reviewed:

PI Rev. 05/99
Rev. 09/99