Cryptosporidium and *Giardia lamblia* Combo Rapid Test Cassette (Feces)

Package Insert

REF ICGC-625	English

A rapid test for the qualitative detection of Cryptosporidium Antigens and Giardia lamblia in human feces.
For professional in vitro diagnostic use only.

INTENDED USE

The *Cryptosporidium and Giardia lamblia* Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of *Cryptosporidium* Antigens and/or *Giardia lamblia* in human feces.

SUMMARY

Cryptosporidiosis is a diarrhoeal disease caused by microscopic parasites of the genus *Cryptosporidium*. Once an animal or person is infected, the parasite lives in the intestine and passes in the feces. The parasite is protected by an outer shell that allows it to survive outside the body for long periods of time and makes it very resistant to chlorine-based disinfectants. Both the disease and the parasite are commonly known as "Crypto." The disease can spread through ingestion of contaminated water or through coughed fomites of an infected individual.[1,2] It can spread by fecal-oral route like other gastrointestinal pathogens.

Parasitary infections remain a very serious health problem worldwide. *Giardia lamblia* is the most common protozoa known to be responsible for one of the main causes of severe diarrhoea in humans, particularly in immunodepressed people. Epidemiological studies, in 1991, showed that infections with Giardia increased in the United States with a prevalence of around 6% on 178,000 samples.[3] Generally, the disease passes through a short acute phase followed by a chronic phase. Infection by *G. Lamblia*, in the acute phase, is the cause of watery diarrhoea with principally the elimination of trophozoites. The feces become normal again, during the chronic phase, with transient emissions of cysts.[4]

The presence of the parasite on the wall of the duodenal epithelium is responsible for a malabsorption. The disappearance of villosities and their atrophy lead to problems with the digestive process at the level of the duodenum and the jejunum, followed by weight loss and dehydration. The majority of infections remain asymptomatic, however. The diagnosis of *G. Lamblia* is carried out under microscopy after flotation on zinc sulphate or by direct or indirect immunofluorescence, on non-concentrated samples displayed on a slide.[5] More and more ELISA methods are also now available for the specific detection of cysts and/or trophozoïtes. Detection of this parasite in surface or distribution water can be undertaken by PCR type techniques.[6]The test is based on the detection of a 65-kDA coproantigen, a glycoprotein that is present in the cysts and trophozoites of *G. Lamblia*.

PRINCIPLE

The *Cryptosporidium and Giardia lamblia* Combo Rapid Test Cassette is a qualitative lateral flow immunoassay for the detection of *Cryptosporidium* antigens and/or *Giardia lamblia* in human feces.

***Cryptosporidium* Antigen Rapid Test Cassette**

The *Cryptosporidium* Antigen Rapid Test Cassette is a qualitative lateral flow immunoassay for the detection of *Cryptosporidium* Antigen in human fecal specimen. The membrane is pre-coated with monoclonal antibodies against *Cryptosporidium* antigens on the test line region. During testing, *Cryptosporidium* antigens, if present in the specimen, bind with anti-*Cryptosporidium* antibodies conjugated particles, which were pre-dried on the test. The mixture moves upward on the membrane by capillary action. In the case of a positive result the specific antibodies present on the membrane will react with the conjugate-antigen complex and generate a colored line in Test Line region. A colored line will always appear in the control line region and serve as verification that sufficient volume was added and proper flow was obtained and as an internal control for the reagents.

***Giardia lamblia* Rapid Test Cassette**

The *Giardia lamblia* Rapid Test Cassette is a qualitative lateral flow immunoassay for the detection of Giardia antigen in human fecal samples. The membrane is pre-coated with monoclonal antibodies against Giardia antigens on the test line region. During testing, the sample reacts with the particle coated with anti-Giardia antibodies, which were pre-dried on the test. The mixture moves upward on the membrane by capillary action. In the case of a positive result, the specific antibodies present on the membrane will react with the mixture conjugates and generate colored lines. A colored line will always appear in the control line region and serve as verification that sufficient volume was added, proper flow was obtained and as an internal control for the reagents.

REAGENTS

The test contains anti-*Cryptosporidium* antibody conjugated, anti-*Giardia lamblia* antibody particles and anti-*Cryptosporidium* antibodies, anti-*Giardia lamblia* antibody coated on the membrane.

PRECAUTIONS

- For professional *in vitro* diagnostic use only.
- Do not use after expiration date.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, do not eat, drink or smoke in the area.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- The used test should be discarded according to local regulations.

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Collect sufficient quantity of feces (1-2 g or 1-2 mL for liquid sample). Fecal specimen should be collected in clean and dry containers (no preservatives or transport media). The samples can be stored in the refrigerator (2-8 ºC) for 1-2 days prior to testing. For longer storage (maximum 1 year), the specimen must be kept frozen below -20 ºC. In this case, the sample will be totally thawed, and brought to room temperature before testing.

MATERIALS

Materials Provided

- Test Cassettes
- Package Insert
- Specimen Collection Tubes with extraction buffer
- Droppers

Materials Required But Not Provided

- Specimen Collection Containers
- Timer

DIRECTIONS FOR USE

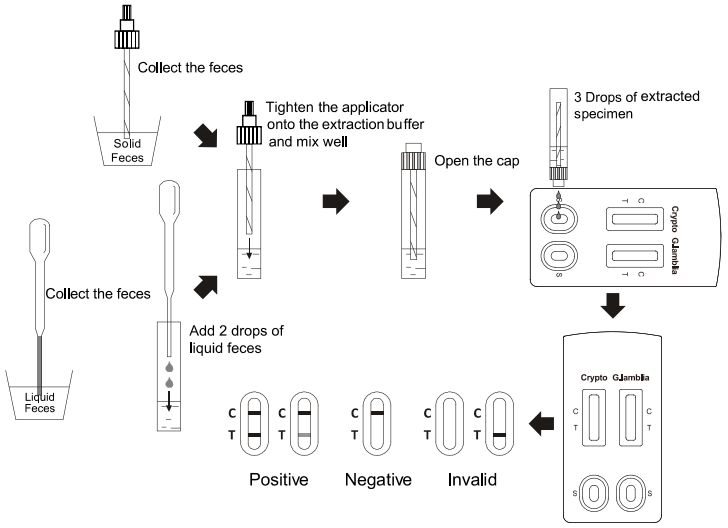
Allow the test cassette, specimen and buffer to reach room temperature (15-30°C) prior to testing.

1. To collect fecal specimens:
Collect sufficient quantity of feces (1-2 mL or 1-2 g) in a clean, dry specimen collection container to obtain enough virus particles. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 1-2 days at 2-8°C. For long term storage, specimens should be kept below -20°C.
2. To process fecal specimens:

- For **Solid Specimens**:
  Unscrew the cap of the specimen collection tube, then randomly **stab the specimen collection applicator into the fecal specimen at least 3 different sites** to collect approximately **50 mg of feces** (equivalent to 1/4 of a pea). Do not scoop the fecal specimen.
- For **Liquid Specimens**:
  Hold the dropper vertically, aspirate fecal specimens, and then transfer **2 drops of the liquid specimen (approximately 80 µL)** into the specimen collection tube containing the extraction buffer.

3. Tighten the cap onto the specimen collection tube, then shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Leave the collection tube for reaction for 2 minutes.
4. Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the test is performed immediately after opening the foil pouch.
5. Hold the specimen collection tube upright and **unscrew the tip** of the specimen collection tube. Invert the specimen collection tube and **transfer 3 full drops of the extracted specimen (approximately 120 µL)** to the specimen well (S) of the test cassette, then start the timer. Avoid trapping air bubbles in the specimen well(S). See illustration.
6. **Read the results at 10 minutes**. Do not interpret the results after 20 minutes.

Note: If the specimen does not migrate (presence of particles), centrifuge the diluted sample contained in the extraction buffer vial. Collect 120 µL of supernatant, dispense into the specimen well(S) of a new cassette. Start the timer and continue from step 6 onwards in the above instructions for use.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

The test results appear in two different test windows respectively for *Giardia lamblia* and *Cryptosporidium* antigens. The interpretation criteria remain the same for positivity or negativity for specific antigens under tests as per indication of the respective Test window. The results are to be interpreted as follows:

POSITIVE: Two colored lines appear. A colored line appears in the Test line region (T) and another colored line appears in the C line region (C).

***NOTE:** The intensity of the color in the test line region (T) will vary depending on the concentration of *Cryptosporidium* antigens or *Giardia lamblia* antigens present in the specimen. However, neither the quantitative value, nor the rate of increase in antigens can be determined by this qualitative test.

NEGATIVE: Only one colored line appears in control line region (C). No line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal valid procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The *Cryptosporidium and Giardia lamblia* Combo Rapid Test Cassette will only indicate the presence of *Cryptosporidium* and/or *Giardia lamblia* antigens in the specimen (qualitative detection) and only should be used for the detection of *Cryptosporidium* antigens and/or *Giardia lamblia* antigens in fecal specimens. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause wrong results. Dilute the sample with the buffer and repeat the test.
3. Do not use specimens treated with solutions containing formaldehyde or its derivatives.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of C*ryptosporidiosis* or *Giardia lamblia.*
5. After one week of infection, the number of parasites in feces is decreasing, making the sample less reactive. Fecal specimen should be collected within one week of the onset of symptoms.
6. This test provides a presumptive diagnosis of *Cryptosporidiosis.* All results must be interpreted together with other clinical information and laboratory findings available to the physician.

PERFORMANCE CHARACTERISTICS

Sensitivity - Specificity

Cryptosporidium Antigen Rapid Test Cassette (Feces) was evaluated on 285 clinical samples (determined by microscopy techniques) from patients in a Hospital.

Method		Microscopic		Total Results
***Cryptosporidium* Antigen Rapid Test Cassette (Feces)**	Results	Positive	Negative	
	Positive	21	6	27
	Negative	1	257	258
Total Results		22	263	285

Relative sensitivity: 95.5% (95%CI*:77.2%~99.9%);
Relative specificity: 97.7% (95%CI*:95.1%~99.2%);
Accuracy: 97.5% (95%CI*: 95.0%~99.0%). *Confidence Intervals

Giardia lamblia Rapid Test Cassette (Feces) was evaluated on 278 clinical samples (determined by microscopy techniques) from patients in a Hospital.

Method		Microscopic		Total Results
***Giardia lamblia* Rapid Test Cassette (Feces)**	Results	Positive	Negative	
	Positive	58	5	63
	Negative	3	212	215
Total Results		61	217	278

Relative sensitivity: 95.1% (95%CI*: 86.3%~99.0%);
Relative specificity: 97.7% (95%CI*:94.7%~99.2%);
Accuracy: 97.1% (95%CI*: 94.4%~98.7%). *Confidence Intervals

Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and negative samples were processed 3 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.

To check inter-batch accuracy (reproducibility), same samples (positive and negative) were processed on kits from three different production batches. All results were confirmed as expected.

Cross-reactivity

For *Cryptosporidium* Antigen Rapid Test Cassette

It was performed an evaluation to determine the cross reactivity of *Cryptosporidium* Antigen test. There is not cross reactivity with other pathogens intestinal virus occasionally present in feces: *Clostridium difficile, E. coli, Giardia Lambia, H. pylori, Salmonella Ifantis, Shigella flexneri, Shigella Sonnei and Shigella dysenteriae* .

For *Giardia lamblia* Rapid Test Cassette

It was performed an evaluation to determine the cross reactivity of *Giardia lamblia* Rapid Test Cassette. There is not cross reactivity with other pathogen intestinal virus occasionally present in feces:
Clostridium difficile, Cryptosporidium parvum, E. Coli, H. pylori, Salmonella Ifantis, Shigella flexneri, Shigella Sonnei and Shigella dysenteriae

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Index of Symbols

	Caution		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2-30°C	LOT	Lot Number	REF	Catalog #
	Do not use if package is damaged		Manufacturer		Consult Instructions For Use

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