JusChek®

Entamoeba/Giardia/Crypto Combo Rapid Test Cassette (Feces) Package Insert

REF IEGC-635 | **English**

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

INTENDED USE

The Entamoeba/Giardia/Crypto Combo Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of *Entamoeba histolytica* antigens, *Giardia lamblia* and *Cryptosporidium* antigens in human feces specimen.

SUMMARY

Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus Entamoeba.[1] Predominantly infecting humans and other primates causing amoebiasis, *E. histolytica*is estimated to infect about 50 million people worldwide. Previously, it was thought that 10% of the world population was infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *E. dispar*.[2] Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission. *E. histolytica*, as its name suggests (histolytic= tissue destroying), is pathogenic; infection can be asymptomatic or can lead to amoebic dysentery or amoebic liver abscess.[1,3]

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 immunedepressed 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.[4] 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.[5]

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.[6] 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.[7] 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*.

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.[8,9] It can spread by fecal-oral route like other gastrointestinal pathogens.

PRINCIPLE

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

Entamoeba histolytica

The *Entamoeba histolytica* Rapid Test is a qualitative, lateral flow immunoassay for the detection of *Entamoeba histolytica* antigen in human feces specimen. The membrane is precoated with anti-*Entamoeba histolytica* antibody on the test line region of the test. During testing, the specimen reacts with the particle coated with an *Entamoeba histolytica* antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-*Entamoeba histolytica* antibody on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Giardia lamblia

The *Giardia lamblia* Rapid Test is a qualitative lateral flow immunoassay for the detection of Giardia antigen in human feces samples. The membrane is pre-coated with 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.

Cryptosporidium

The Cryptosporidium Antigen Rapid Test is a qualitative lateral flow immunoassay for the detection of Cryptosporidium antigen in human feces samples. The membrane is pre-coated with 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.

REAGENTS

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

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after the expiration date.
- The test should remain in the sealed pouch until use.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.

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

The feces must be collected in clean, dry, waterproof container containing no detergents, preservatives or transport media.
Bring the necessary reagents to room temperature before use.
If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

MATERIALS

Materials Provided

- Test Cassettes
- Package Insert
- Specimen collection tubes with extraction buffer
- Droppers

Materials Required But Not Provided

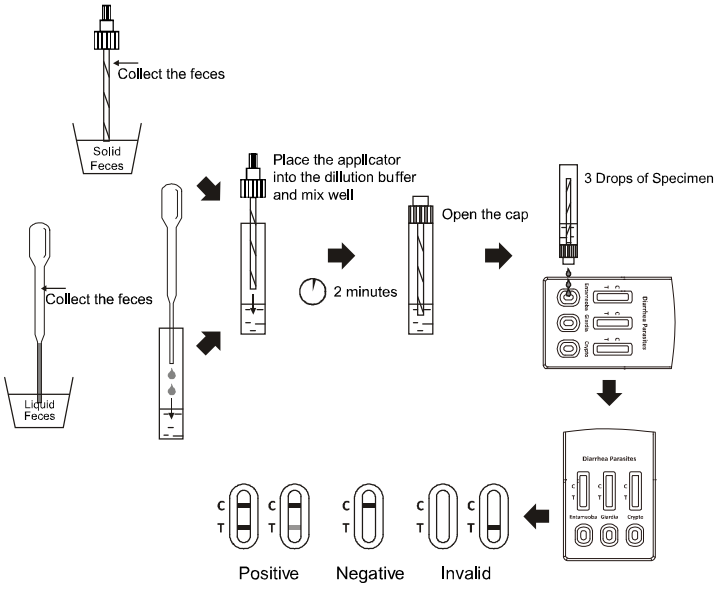
- Specimen collection containers
- Timer

DIRECTIONS FOR USE

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

1. To collect fecal specimens:
   Collect sufficient quantity of feces (1-2mL or 1-2g) in a clean, dry specimen collection container to obtain enough pathogens. Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected may be stored for 3 days at 2-8°C if not tested within 6 hours. 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 below.
6. Read the results at **10 minutes** after dispensing the specimen. Do not read 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 three different test windows respectively for *Entamoeba histolytica* antigens, *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.** One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

***NOTE**: The intensity of the color in the test line region will vary depending on the concentration of Entamoeba histolytica antigens, Giardia lamblia and Cryptosporidium antigens present in the specimen. Therefore, any shade of color in the test line region should be considered positive.

NEGATIVE: **One colored line appears in the 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 *Entamoeba/Giardia/Crypto* Combo Rapid Test Cassette will only indicate the presence of *Entamoeba* and/or *Giardia lamblia* and/or *Cryptosporidium* antigens in the specimen (qualitative detection) and only should be used for the detection of *Entamoeba* antigens and/or *Giardia lamblia* antigens and/or *Cryptosporidium* antigens in feces 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 *Entamoeba histolytica, Giardia lamblia and* Cryptosporidium.
6. As with all diagnostic tests, all results must be considered with other clinical information available to the physician

EXPECTED VALUES

The Entamoeba/Giardia/Crypto Combo Rapid Test has been compared with Other Rapid Test. The correlation of *Entamoeba histolytica* between the two systems is 99.0%. The correlation of *Giardia lamblia* between the two systems is 96.9%. The correlation of *Cryptosporidiosis* between the two systems is 97.8%.

PERFORMANCE CHARACTERISTICS

Sensitivity - Specificity

The *Entamoeba/Giardia/Crypto Combo* Rapid Test Cassette (Feces) was evaluated on 288 patients of *Entamoeba histolytica*.

Method		**Other Rapid Test**		**Total Result**
Entamoeba/Giardia/Crypto Combo Rapid Test Cassette (Feces)	Results	Positive	Negative	
	Positive	42	2	44
	Negative	1	243	244
Total Results		43	245	288

Relative sensitivity: 97.7% (95%CI*: 87.7%~99.9%);
Relative specificity: 99.2% (95%CI*:97.1%~99.9%);
Accuracy: 99.0% (95%CI*: 97.0%~99.8%). *Confidence Intervals

The *Entamoeba/Giardia/Crypto Combo* Rapid Test Cassette (Feces) was evaluated on 318 patients of *Giardia lamblia*.

Method		**Other Rapid Test**		**Total Result**
Entamoeba/Giardia/Crypto Combo Rapid Test Cassette (Feces)	Results	Positive	Negative	
	Positive	69	6	75
	Negative	4	239	243
Total Results		73	245	318

Relative sensitivity: 94.5% (95%CI*: 86.6%~98.5%);
Relative specificity: 97.6% (95%CI*:94.7%~99.1%);
Accuracy: 96.9% (95%CI*: 94.3%~98.5%). *Confidence Intervals

The *Entamoeba/Giardia/Crypto Combo* Rapid Test Cassette (Feces) was evaluated on 275 patients of *Cryptosporidium*.

Method		**Other Rapid Test**		**Total Results**
Entamoeba/Giardia/Crypto Combo Rapid Test Cassette (Feces)	Results	Positive	Negative	
	Positive	29	5	34
	Negative	1	240	241
Total Results		30	245	275

Relative sensitivity: 96.7% (95%CI*: 82.8%~99.9%);
Relative specificity: 98.0% (95%CI*:95.3%~99.3%);
Accuracy: 97.8% (95%CI*: 95.3%~99.2%). *Confidence Intervals

Repeatability and reproducibility

To check intra-batch accuracy (repeatability), the same positive samples and negative sample 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

Cross reactivity with following organisms has been studied at 1.0E+07 organisms/ml. The following organisms were found negative when tested with the *Entamoeba/Giardia/Crypto* Combo Rapid Test Cassette (Feces):

H.pylori,	*Clostridium difficile*	*Salmonella Ifantis*
Shigella flexneri	*Shigella Sonnei*	*Shigella dysenteriae*
E.coli		

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Index of Symbols

	Attention, see instructions for use		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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CE

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