FRENOVO

Crypto-Giardia-Entamoeba Combo Rapid Test Kit

Instructions For Use

PRODUCT NAME

Crypto-Giardia-Entamoeba Combo Rapid Test Kit

PACKAGE SPECIFICATION

25 tests/kit

INTENDED USE

Crypto-Giardia-Entamoeba Combo Rapid Test Kit is an in vitro qualitative immunochromatographic assay for the rapid detection of Cryptosporidium, Giardia , Entamoeba (histolytica and/ordispar) in stool samples. The test offers a simple and highly sensitive screening assay to make a presumptive diagnosis of cryptosporidiosis and/or giardiasis and/or amoebiasis.

SUMMARY AND PRINCIPLES OF THE PROCEDURE

Cryptosporidium parvum, Giardia lamblia and Entamoeba histolytica are three of the major causes of protozoan-induced diarrheal disease and are the most frequently identified protozoan parasites causing waterborne disease outbreaks. Giardia causes an intestinal illness called giardiasis. Cryptosporidium is responsible for a similar illness called cryptosporidiosis. And amoebiasis is the infection of the human gastrointestinal tract by Entamoeba histolytica. These infections have become the most common causes of waterborne diseases (found in both drinking and recreational water) in humans.

Giardia, a flagellated protozoan, inhabits the upper part of the small intestine of its host and has a two major states in the life cycle: trophozoites which produces the antigens (α-1 giardin) and cyst with produces the antigens (CWP1) After the host ingest the cysts, which are the infective stage, the trophozoites emerge from the cysts in the duodenum and attach to the small intestinal mucosa. They undergo mitotic division in the intracellular lumen, some will encyst to protect themselves and will be eliminated from the host in the feces. The trophozoite is the vegetative form and replicates in the small intestine.

Giardiasis is a diarrheal illness caused by a very small parasite, Giardia intestinalis (also known as Giardia lamblia and Giardia duodenalis). Once an animal or person is infected with Giardia, the parasite lives in the intestine and is passed in the stool. The parasite is protected by an outer shell and can survive outside the body and in the environment for a long time. The most common symptoms of giardiasis include: diarrhea, loose or watery stool, stomach cramps and upset stomach. These symptoms generally begin 1-2 weeks after infection and may last 2-6 weeks in healthy individuals. Sometimes symptoms last longer and may lead to weight loss and dehydration. Some people will have no symptoms. However, people with weakened immune systems (e.g., persons with HIV/AIDS, cancer patients and transplant patients) or the elderly may have a more serious infection that can lead to severe illness or death.

Several species and genotypes of Cryptosporidium have been associated with human infections, although C. hominis (previously known as the human genotype of C. parvum) and C. parvum have been identified as the chief causes of human cryptosporidiosis. The disease is more common in young children and immunosuppressed patients, in whom it can be very serious, but it can also affect healthy people of any age. However, in immunocompetent patient, clinical manifestations of cryptosporidiosis range from the absence of symptoms, though mild symptoms, to a severe gastroenteritis-like syndrome with watery diarrhoea. Cryptosporidium parvum is the major cause of persistent diarrhoea in developing countries. This parasite is recognised as a highly infectious enteric pathogen and infective stage is transmitted by the fecal-oral route. Symptoms of cryptosporidiosis include watery diarrhoea, stomach cramps, weight loss, nausea and sometimes fever. The incubation period is 5 to 28 days with a mean of 7.2 days. Other symptoms include abdominal pain and fatigue.

Entamoeba histolytica, E. dispar, and E. moshkovskii are morphologically identical but biochemically and genetically different. These parasites colonize the human gut, but only E. histolytica is thought to be capable of causing disease. Amoebiasis is the infection of the human gastrointestinal tract by Entamoeba histolytica, a protozoan parasite that is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver. The acceptance of E. dispar as a distinct but closely related protozoan species has had profound implications for the epidemiology of amoebiasis, since most asymptomatic infections found worldwide are now attributed to this non invasive amoeba. Roughly, 90% of people infected with Entamoeba infections have E. dispar. The disease may manifest itself as an acute, chronic or as an asymptomatic infection. The leading symptom is dysentery whose main consequence one galling of the colon, diarrhoea and abdominal pain. Complications of the disease may be hepatic abscesses, pulmonary abscesses or even cerebral abscesses which, if untreated, usually end in death.Infection occurs by water pollution, vegetables, raw fruits and other food or poorly cooked or washed with infective cysts from contaminated faeces. It is possible that flies and cockroaches carrying cysts from faeces to food Amoebic dysentery occurs frequently in tropical countries but are also cases in temperate and cold areas. In Africa, tropical Asia and Latin America, more than two thirds of the population has these intestinal parasites, although most can be virtually asymptomatic infections.

Crypto-Giardia-Entamoeba Combo Rapid Test is a sandwich solid phase immunochromatographic assay. To perform the test, an aliquot of diluted stool sample is added to the sample well of the test cassette. The sample flows through a pad containing antibodies against Crypto, Giardia and Entamoeba coupled to red-colored colloidal gold. If the sample contains Crypto , Giardia, or Entamoeba antigens, the antigen will bind to the antibody coated on the colloidal gold particles to form antigen-antibody-gold complexes. These complexes move on the nitrocellulose membrane by capillary action toward the test line region on which Crypto, Giardia and Entamoeba specific antibodies are immobilized separately. As the complexes reach the test line, they will bind to the antibody corresponding to the parasite antigen on the membrane to form of a line. A red control line will always appear in the result window to indicate that the test has been correctly performed and the test device functions properly. If Crypto , Giardia, or Entamoeba antigen is not present or lower than the detection limit of the test, only the control line will be visible. If the control line dose not developed, the test is invalid.

KIT COMPONENTS

Each kit contains:

1. Test devices: 25 pieces test devices individually pouched.
2. Sample collection tubes: 25 pieces tubes and 1.0 ml collection solution in each tube.
3. Instructions For Use: 1 piece attached.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or stopwatch.
- Biohazard disposal waste container.
- Disposable gloves and/or protective clothing.

WARNINGS

1. Read the Instructions For Use completely before using the product. The instructions must be followed carefully as not doing so may result in inaccurate results.
2. The kit is for diagnostic use only.
3. Perform test at room temperature.

PRECAUTIONS

1. The kit is for professional use only.
2. The Instructions For Use instructions must be followed to ensure optimum test performance.
3. The kit is intended for in vitro diagnostic use.
4. As with all screening assays, any results should be considered presumptive until confirmatory assays have been performed according to local practice or WHO guidelines.

Safety Precautions

1. Standard precautions for handling infectious agents should be observed when using this kit.
2. Wear protective clothing such as lab coat, safety glasses and disposable gloves when handling specimens and assay reagents.
3. Wash hands thoroughly after use.
4. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Bio safety Precautions

Appropriate bio safety practices should be used when handling specimens and reagents. These precautions include, but are not limited to the following:

1. Do not smoke, eat, drink, apply cosmetics or handle contact lenses in areas in which specimens are handled.
2. Dispose of all specimens, used devices and tubes as though they are capable of transmitting infection. The preferred methods of disposal are by autoclave at 121℃ for a minimum of 60 minutes or by incineration. Disposable materials may be incinerated. Liquid waste may be mixed with appropriate chemical disinfectants. A solution of 10% bleach is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions containing bleach.
3. When disposing of sample collection tubes, avoid contact with acid to prevent liberation of a toxic gas.
4. All spills should be wiped thoroughly using a suitable disinfectant such as a sodium hypochlorite solution.
5. Use a separate tube and device for each specimen tested.

Handling Precautions

1. Do not use if the kit box safety seal is absent, damaged or broken.
2. Do not use any device if the pouches have been perforated.
3. Each device is for single use only.
4. Do not mix sample collection tubes/test devices from different kit lots.
5. Do not use the kit past the expiration date (this date is printed on the kit box).
6. Adequate lighting is required to read the test results.
7. The result should be read immediately after the end of the 10 minutes incubation time following the addition of collected specimen. Do not read results beyond 15 minutes.

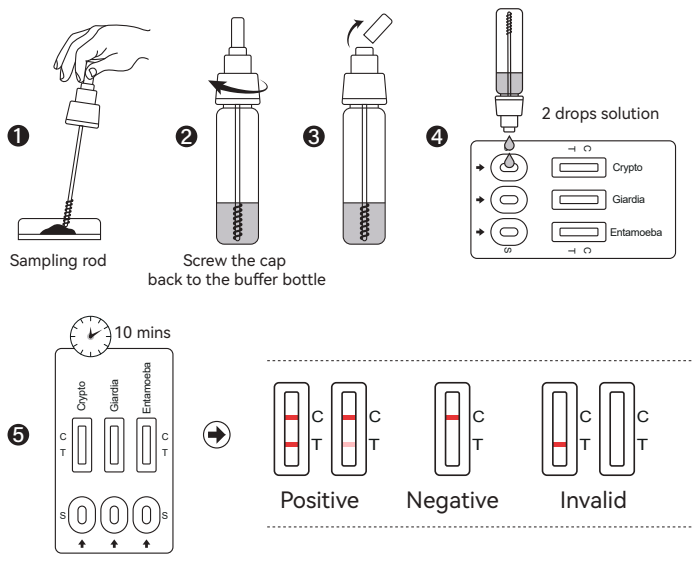
STORAGE INSTRUCTIONS

1. The kit should be stored between 2-30°C and the shelf life is 24 months.
2. The kit components are stable until the expiration date printed on the outer label, when stored as directed. The kit expiry date is determined by whichever of the components has the shortest expiry date. The kit expiry date is not impacted once the extraction Solution has been opened. Do not use kit components beyond overall kit expiry date.
3. If stored refrigerated, ensure that the pouched device is brought to room temperature before opening.
4. Do not freeze the kit.

TEST PROCEDURE

Allow the test device, specimen, extraction solution to equilibrate to room temperature (15-30°C) prior to testing.

1. Loosen the sample collection tube, take out the sampling rod, insert the feces sample, pick up 50 mg fecal sample (equivalent to the size of match head) and then put the sampling rod back into the tube, and rotate it tighten and shake well.
2. Place the test cassette on a clean and level surface. Break the tip of the sample collection tube, abandon the first two drops and draw two drops (approximately 90ul) into the Crypto/-Giardia/ Entamoeba sample well respectively, then start the timer. Avoid trapping air bubbles in the sample well.
3. Wait for the colored line(s)to appear. The test result should be read at 10 minutes. Do not interpret the result after 15 minutes.



INTERPRETATION OF RESULTS

Negative result: if there is only a quality control line C, the detection line is colorless, indicating that Crypto/Giardia/ Entamoeba antigen has not been detected and the result is negative.

Positive result: Two lines appear on test strip. One colored line should be in the control line region(C), and one colored lines should appear in test line region(T) . The color intensities of the lines do not have to match, indicating that Crypto/Giardia/ Entamoeba antigen has been detected

Invalid result: if the quality control line C is not observed, it will be invalid regardless of whether there is detection line (as shown in the figure above), and the test shall be conducted again.

LIMITATIONS

1. The kit is for professional in vitro diagnostic use only. The test should be used for the detection of Crypto/Giardia/ Entamoeba antigen in human feces samples. Neither the quantitative value nor the rate of increase in Crypto/Giardia/ Entamoeba antigen can be determined by this qualitative test.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. A negative result may be obtained if the concentration of the Crypto/Giardia/ Entamoeba antigen present in The specimen is not adequate or is below the detectable level of the test.
4. The accuracy of the test depends on the quality of the sample, false negatives may result from improper sample collection or storage.
5. After one week of infection, the number of parasites in faeces is decreasing, making the sample less reactive. Stool samples should be collected within one week of the onset of symptoms.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity
Clinical study was performed to compare the results obtained by The kit and qPCR. The results indicated that The kit has a high sensitivity and specificity as summarized below:

Crypto clinical study		qPCR		
Crypto-Giardia-Entamoeba Combo Rapid Test	Results	Positive	Negative	Total Results
	Positive	32	1	33
	Negative	2	89	91
Total Results		34	90	124

Clinical sensitivity=94.12% (95%CI * 80.8 – 99.3%)
Clinical specificity=98.89% (95%CI * 96.0–99.7%)
Accuracy=97.58% (95%CI * 92.61% to 99.66%)

Giardia clinical study		qPCR		
Crypto-Giardia-Entamoeba Combo Rapid Test	Results	Positive	Negative	Total Results
	Positive	45	3	48
	Negative	1	76	77
Total Results		46	79	125

Clinical sensitivity=97.83% (95%CI * 88.2–99.9%)
Clinical specificity=96.20% (95%CI * 91.4–99.7%)
Accuracy=96.80% (95%CI * 95.26% to 99.66%)

Entamoeba clinical study		qPCR		
Crypto-Giardia-Entamoeba Combo Rapid Test	Results	Positive	Negative	Total Results
	Positive	19	2	21
	Negative	7	214	221
Total Results		26	216	242

Clinical sensitivity=73.08% (95%CI * 52.2 - 88.4%)
Clinical specificity=99.07% (95%CI * 96.7 - 100%)
Accuracy=96.28% (95%CI * 95.26% to 99.66%)

Cross Reaction
An evaluation was performed to determine the cross reactivity of Crypto-Giardia-Entamoeba Combo Rapid Test ; no cross reactivity against gastrointestinal pathogens, other organism, substances and/or faecal markers occasionally present in faeces:

For Crypto:

Adenovirus	Pig Haemoglobine
Entamoeba histolytica	Shigella sonnei
Legionella pneumophila	Campylobacter jejuni
Salmonella typhimurium	Helicobacter pylori
Astrovirus	Rotavirus
Escherichia coli O157	Streptococcus pyogenes
Listeria monocytogenes	Clostridium difficile antigen GDH
Shigella boydii	Human Haemoglobine
Bovine Haemoglobine	Salmonella enteritidis
Escherichia coli O:111	Streptococcus pneumococcal
Norovirus GI	Clostridium difficile Toxin A
Shigella dysenteriae	Human Lactoferrin Salmonella paratyphi A
Calprotectin	Yersinia Enterocolitica O:3
Escherichia coli O:026	Clostridium difficile Toxin B
Norovirus GII	Human Transferrin
Shigella flexneri	Salmonella typhi
Campylobacter coli	Yersinia Enterocolitica O:9
Giardia	Clostridium perfringens

For Giardia:

Adenovirus	Pig Haemoglobine
Cryptosporidium parvum	Shigella sonnei
Legionella pneumophila	Clostridium difficile antigen GDH
Salmonella typhimurium	Helicobacter pylori
Astrovirus	Rotavirus
Entamoeba histolytica	Streptococcus pyogenes
Listeria monocytogenes	Clostridium difficile Toxin A
Shigella boydii	Human Haemoglobine
Bovine Haemoglobine	Salmonella enteritidis
Escherichia coli O157	Streptococcus pneumococcal
Norovirus GI	Clostridium difficile Toxin B
Shigella dysenteriae	Human Lactoferrin
Calprotectin	Salmonella paratyphi A
Escherichia coli O:111	Yersinia Enterocolitica O:3
Norovirus GII	Clostridium perfringens
Shigella flexneri	Human Transferrin
Campylobacter jejuni	Salmonella typhi
Escherichia coli O:026	Yersinia Enterocolitica O:9

For Entamoeba:

Campylobacter jejuni	Shigella flexneri
Helicobacter pylori	Escherichia coli O157:H7
Shigella boydii	Salmonella paratyphi
Campylobacter coli	Shigella sonnei
Listeria monocytogenes	Cryptosporidium parvum
Shigella dysenteriae	Salmonella typhi
Clostridium difficile	Staphylococcus aureus
Salmonella enteritidis	Giardia lamblia
	Salmonella typhimurium

INDEX OF SYBOML

IVD	In vitro diagnostic medical device		single-use, Please don't reuse it
	Use-by date		Consult instructions for use
	Cautions		Manufacturer
2°C 30°C	Temperature limit	LOT	Batch code
	Date of manufacture		Keep Dry
	Avoid overexposure to the sun		Don't use the product when the package is damaged
	Biological risks		

Hangzhou Frenovo Biotech Co., Ltd.
Address: Room 401,Building 26, Room 401&301,Building 27,No.488-1,Donghu North Road, Donghu Street,LinPing District,Hangzhou City,Zhejiang Province. China.
Tel: +86-571-89170657
Email: business@frenovo.com

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