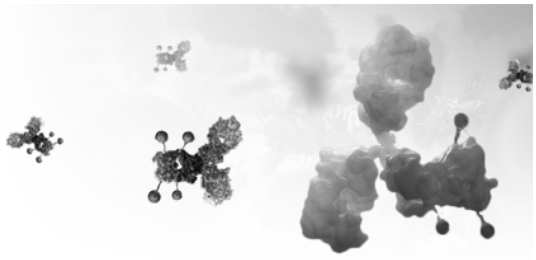

Crypto-Giardia-Entamoeba Rapid Test (DTS700)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Intended Use	For in vitro diagnostic use. Crypto-Giardia-Entamoeba Rapid Test is an immunochromatographic rapid assay for the qualitative determination of Cryptosporidium parvum and/or Giardia lamblia and/or Entamoeba histolytica (sensu lato) in stool samples.
General Description	##Giardia lamblia## is an intestinal flagellate. The morphologically characteristic Trophozoites only survive for a short time outside the host organism. Transmission takes place via the highly infectious cysts. Because it is spread world- wide, Giardia lamblia has become an important cause of chronic diarrhoeas, particularly in the case of problems in emporiatrics. The infection occurs after the ingestion of cysts in contaminated food and water. In communal facilities with inadequate hygiene, the infection usually occurs via the faecal-oral route from person to person. This mode of transmission is particularly common among children and in kindergartens, as well as among male homosexuals and prison inmates. The infection can also be passed on from children to parents. Unlike infants, older children who are infected can be free of the symptoms. Nevertheless, they excrete the cysts and can infect other humans. The symptoms of Giardiasis (Lambliasis) are acute or chronic diarrhoea. The incubation time is between 3 and 42 days. The method most frequently used to diagnose Giardiasis in the past has been the detection of cysts in the stool by microscopy, which can only be carried out by experienced personnel. The investigations also have to be carried out over a long period of time because the excretion of cysts fluctuates greatly. ##Cryptosporidium parvum## is a parasite which is very common in animals and occurs as an important pathogenic organism in domestic animals and particularly in calves. However, infections in humans are now observed in many countries more frequently than was previously assumed. In tropical developing countries, the parasite is often endemic and causes diarrhoea epidemics among children. With immunocompetent patients, the disease manifests itself as self-healing gastroenteritis. The diarrhoea lasts between 3 and 10 days and may be accompanied by fever and gastrointestinal symptoms such as nausea and pain, which resembles those of giardiasis (lambliasis). The symptoms and effects are substantially more serious with immunoincompetent patients, where diarrhoea persists and is very severe. The infection can be transmitted from animal to humans via contaminated water and from human to

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	human. Members of communal facilities, children in kindergartens and the high-risk groups, homosexual men and patients infected with HIV, are particularly at risk. In the past, the methods most frequently used for the diagnosis of cryptosporidiosis were the microscopic detection of Oocysts in the stool or the microscopic examination of small intestine biopsy samples which can only be carried out by experienced personnel. Across the world, up to 500 million people are infected with ##Entamoeba histolytica (sensu lato)## every year. Molecular-genetic investigations have shown that the protozoa, which has been given the name Entamoeba histolytica and is identified using conventional diagnostic methods, consists of two morphologically indistinguishable species: the pathogenic species, Entamoeba histolytica sensu stricto and the non-pathogenic species (according to current knowledge), Entamoeba dispar. Roughly 90% of people with Entamoeba infections have E. dispar. The approximately 40-50 million cases of amoebic colitis or hepatic abscess which result in 80,000 deaths every year are caused by E. histolytica. The life cycle of the Entamoeba is relatively straightforward. The infection is caused by the oral ingestion of cysts with four nuclei. In the small intestine, these develop into the single nucleus form of the parasite, the trophozoite (forma minuta), which multiplies and differentiates predominantly in the large intestine. Encapsulation is probably triggered by the environment in the lower region of the large in testine. Besides the cysts, trophozoites are only found in stools with accelerated intestinal passage. The clinical symptoms of amoebiasis are triggered by the invasion of the parasite from the lumen of the bowels into the mucous membrane of the colon. Trophozoites with phagocytised erythrocytes are frequently found at the same time. These trophozoites are known as forma magna because of their size. The symptoms of invasion into the mucous membrane of the intestine are diarrhoea, dysentery or even amebomas. The complications which may occur after disseminate dispersion are hepatic abscesses, pulmonary abscesses or, in very rare cases, even cerebral abscesses which, if untreated, usually end in death.
Reagents And Materials Provided	There are enough reagents in the pack for 20 determinations. 1.##Cassette##: 20 individually packed test cassettes. 2.##Diluent##: 26 ml; Extraction buffer, ready for use; contains 0.1 % sodium azide. 3.##Pipet##: Bag containing 25 disposable pipettes
Materials Required But Not Supplied	1. Test tubes for stool suspension 2. Vortex mixer (optional) 3. Micropipette (200 µl - 1000 µl) 4. Waste container containing 0.5% sodium hypochlorite solution
Storage	The pack can be stored at 2-30°C and can be used until the printed expiry date. After the expiry date, the quality guarantee is no longer valid. Likewise, the usability of the cassettes cannot be guaranteed once the external packaging of the individual cassette has been damaged.
Specimen Collection And Preparation	Stool samples must be collected in clean containers without any additives and stored at 2 - 8°C before beginning the test. If stored for more than 3 days, the sample must be frozen at -20°C. In this case, the sample must be completely thawed out and brought to room temperature before

	testing begins. Avoid freezing and thawing the sample repeatedly. If rectal swabs have to be used, make sure that sufficient stool material (approx.50 mg) is collected to carry out the test.
Reagent Preparation	The samples, extraction buffer and test cassettes must be brought to room temperature (20-25°C) before using. The test cassettes must only be removed from the external packaging shortly before they are used. Once used, the cassettes must not be used again. The test must not be carried out in direct sunlight. Do not pour reagents back into vials since this may lead to contamination of the reagent.
Assay Procedure	##Preparing the samples## Place 1 ml Extraction Buffer Diluent in a labelled test tube. With the liquid stool sample, pipette 100 µl (up to just above the second thickening) of the sample and suspend it in the buffer which was placed in the tube beforehand. With solid stool samples, suspend 50 mg of the sample in the buffer. The sample must then be well homogenised. This can be achieved either by repeated suction and ejection of the suspension using the disposable pipette Pipet or, alternatively, by mixing on a vortex mixer. Afterwards, allow the homogeneous suspension to settle for at least 3 minutes until a clear supernatant is formed. ##Testing the sample ## When removed from the external packing, first lay the test cassette on a level mat. After this, pipette 200 µl(micropipette) or 4 drops (disposable pipette) of the clear supernatant of the stool suspension into the round opening of the test cassette. Make sure that the liquid flows through the membrane unimpeded. Any particles pipetted at the same time can cause an obstruction and must be removed beforehand. The test result can be read off after 10 minutes.
Quality Control	The test must only be evaluated if the test cassette is intact before the sample suspension is pipetted into it and no colour changes or bands are visible on the membrane. In addition to this, at least the crimson control band must be visible after the test incubation. If this does not appear, the following must be check ed before repeating the test: - Expiry date of the test cassettes and the extraction buffer being used - Correct test procedure - Contamination of the extraction buffer If the control band is still not visible after repeating the test with a new test cassette, please contact us.
Interpretation Of Results	A maximum of four bands should appear in the following order, as seen from the sample-application site: one blue ("1" = Test band 1), one red ("2" = Test band 2), one green ("3" = Test band 3) and one crimson (C = control band) band. ##If the crimson control band is missing, the test is invalid and cannot be evaluated!## The following interpretations are possible: - Cryptosporidia positive: blue and crimson bands are visible. - Giardia positive: red and crimson bands are visible. - Entamoeba positive: green and crimson bands are visible. The three specific test bands may also appear in any combination with the crimson control band, depending on which of the three pathogens are present in the sample.

- Negative: only the crimson control band appears.
- Not valid: no visible band or a combination other than the one described above or other changes in band colour. Likewise, any changes in band colour which appear after 10 minutes or later are also without any diagnostic value and must not be used for evaluation.

Performance Characteristics

In a multi-centre study involving five different institutions, a total of 252 stool samples (which had been determined beforehan d using different methods and kept frozen for later use) were thawed and analysed using the Cryptosporidium/Giardia/Entamoeba Combi rapid assay. The individual results are listed in Table 1. The average sensitivity and specificity have been calculated from the individual results from the five validation centres.

Table 1 Results from a multi-centre study using the Cryptosporidium/Giardia/Entamoeba Combi rapid assay

Reference method	Samples				Specific Parasite Test Band					
	total	pos.	negative		Cryptosporidium		Giardia		Entamoeba	
			no parasites	other	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
Microscopy	**28**	28	0	0	87.5	-	80	-	60	-
Microscopy	**63**	32	20	11	100	100	100	100	-	100
Microscopy	**32**	12	15	5	-	-	88.9	100	100	80
Microscopy / PCR	**49**	35	5	9	66.7	79.9	94.4	100	79.2	76
Elisa	**80**	63	17	0	77.8	100	96.3	98.1	100	93.6
Total	**252**	**170**	**57**	**25**	**83.0 %**	**93.3%**	**91.9%**	**99.5%**	**84.8%**	**87.4%**

Precautions

For in vitro diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test strictly adhered to.
The extraction buffer contains sodium azide as a preservative. These substances must not be allowed to come into contact with the skin or mucous membrane.
Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples are being used.
All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectants (e.g. sodium hypochlorite) in exactly the same way as the samples themselves or autoclaved for at least one hour at 121°C.

Limitations

Cryptosporidium/Giardia/Entamoeba Combi rapid test detects the antigens of Cryptosporidium parvum and / or Giardia lamblia and / or Entamoeba histolytica (sensu lato) in stool samples.
The test cannot be used to derive a relationship between the intensity of the specific visible bands and the occurrence or severity of clinical symptoms. The results obtained must always be interpreted in combination with the clinical picture.
##A positive## result does not rule out the presence of another infectious pathogen.

##A negative## result does not necessarily mean that there is no infection with Cryptosporidiae, Lambliae or Entamoebae. This can be due to intermittent excretion of the pathogen or to the quantity of antigens in the sample being too small. If the patient is anaemic or is suspected as being infected by the pathogens being looked for, another stool sample should be tested after four weeks.

An excess of stool sample can cause brownish bands to appear instead of the specifically coloured bands. These brownish bands do not have any diagnostic value. In such cases, it will be necessary to repeat the test with a smaller stool quantity or dilute the suspension already prepared further (clear supernatant after precipitation) in order to clarify whether the pathogens being looked for are in the sample and have been masked by too much stool matrix.

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