RIDA®QUICK
Cryptosporidium/Giardia/Entamoeba Combi

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Cryptosporidium/Giardia/Entamoeba Combi 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.

2. Summary and explanation of the test

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 particular 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 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 intestine. 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.

The clinical symptoms of the acute intestinal form of amoebiasis are cramp-like abdominal pains with nausea and severe diarrhoea with bloody and slimy stools. The acute stage can develop into a chronic stage with occasional diarrhoea alternating with constipation, abdominal pain, nausea and vomiting. Completely symptom-free cyst elimination has also been described.

Approximately 10% of cases of acute amoebic dysentery result in extra-intestinal complications such as hepatic abscesses or the invasion of other organs. With extra-intestinal amoebiasis, a serological determination of antibodies is indicated.

Intestinal amoebiasis can be diagnosed by using complicated microscopic procedures for determining the cysts and trophozoites in the stools. However, since the parasite density can be very small, it can be assumed that the sensitivity of this method for a single stool investigation is only 75%, even with experienced personnel. The method also involves the risk of confusing Entamoeba with cells of the intestine epithelium, granulocytes, macrophages and fungi.

Sensitive immunological test procedures with specific antibodies against antigens of Entamoeba have a great advantage. The diagnostic method is not dependent on subjective evaluation and is more sensitive since components which can no longer be identified by their morphology are

also used in the determination. Only the invasive form of Entamoeba causes the formation of antibodies. Since antibody titers can usually be detected with the onset of the clinical symptoms, a specific antibody determination can be used to identify E. histolytica. This also offers the possibility of differentiating between the size of the titers of intestinal and extra-intestinal amoebiasis, which is crucial for deciding on the choice of therapy.

An important alternative method to microscopy for the detection of Giardia lamblia and Cryptosporidium parvum is the quick immunochromatographic test described in the following which, because it uses monoclonal antibodies, is equivalent to the microscopy investigation procedures in terms of sensitivity and specificity. The test is quick and simple to perform and does not require specially trained microbiologists to carry it out.

3. Test principle

This rapid assay is a single-step immunochromatographic lateral-flow test, where specific antibodies which are directed against each parasite attach themselves to green (Entamoeba specific), red (Giardia specific) or blue (Cryptosporidia specific) latex particles. Other specific antibodies against the three pathogens are firmly bound to the membrane. The stool sample is first suspended in the extraction buffer and then precipitated. The test strip is dipped in the clear supernatant of the sample. The sample then passes, with the coloured latex particles, to which the antigen is attached if present if the test is positive, through the membrane and bonds to the specific collection bands. A green and/or red and/or blue band appears, depending on the antigens present in the sample.

4. Reagents provided

There are enough reagents in the pack for 25 determinations.

Strip	25 det.	Tube with 25 test strips
Diluent	26 ml	Extraction buffer, ready for use; contains 0.1 % sodium azide
Pipet	25 pieces	Bag containing 25 disposable pipettes

5. Storage instructions

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. The tube with the test strips contains a drying agent and, in order to prevent the strips from getting damp, must not be left open. It must be carefully closed again each time a test strip is removed.

6. Materials required but not provided

Test tubes for stool suspension
Tubes (optional: uncoated microtiter wells) for the suspension supernatant
Vortex mixer (optional)
Micropipette (200 µl - 1000 µl)
Waste container containing 0.5% sodium hypochlorite solution

7. Precautions for users

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 sample dilution 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.

8. Sample collection and storage

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.

9. Test procedure

9.1. General information

The samples, extraction buffer and the test strips must be brought to room temperature (20-25°C) before use. The tube with the test strips must not be opened until it has reached room temperature and must be closed again after removing the necessary test strips. Once used, the test strips 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.

9.2. Preparing the samples

Place 1 ml Extraction Buffer Diluent in a labelled test tube. With the **liquid** stool sample, pipette Pipet 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. After this, allow the homogenized suspension to precipitate for at least **3 minutes** until a clear supernatant is formed from which at least **200 µl** but at most **500 µl** is then transferred into another clean tube (or uncoated microtiter well).

9.3. Testing the sample

Remove the test strip Strip from the tube and immerse it in the prepared sample. The test strip must not be immersed any further than the line indicated by the arrow. The test result can be read off after **10 minutes**.

10. Quality control – signs of reagent expiry

The test must only be evaluated if the test strip is intact and no colour changes or bands can be seen on it **before** immersing it in the sample suspension which has been prepared. 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 checked before repeating the test:

- Expiry date of the test strips 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 strip, please contact the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

A maximum of four bands should appear in the following order, as seen from the sample-absorption site: One blue, one red, one green and one crimson (control) 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.

12. Limitations of the method

RIDA®QUICK Cryptosporidium/Giardia/Entamoeba Combi 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.

13. Performance characteristics

13.1. Clinical comparison study

In a multi-centre study involving five different institutions, a total of 252 stool samples (which had been determined beforehand using different methods and kept frozen for later use) were thawed and analysed using the RIDA®QUICK 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 RIDA®QUICK Cryptosporidium/Giardia/Entamoeba Combi rapid assay

Reference method	Samples				Specific Parasite Test Band					
	total	pos.	negative		**Cryptosporidium**		**Giardia**		**Entamoeba**	
			no parasites	other parasites	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%**

13.2. Cross reactivity

None of the following specified intestine parasites led to a cross reaction in RIDA®QUICK Cryptosporidium/Giardia/ Entamoeba Combi:

Entamoeba coli
Blastocystis hominis
Chilomastix mesnili
Endolimax nana
Entamoeba nana
Entamoeba hartmannii
Hymenolepsis nana
Isospora belli
Isospora felis
Jodamoeba buetschlii

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