RIDA®QUICK Entamoeba

REF N1703

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1. Intended use

For *in vitro* diagnostic use. RIDA®QUICK Entamoeba is an immunochromatographic rapid assay for the qualitative determination of *Entamoeba histolytica* (sensu lato) in stool samples.

2. Summary and explanation of the test

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 have been given the name *Entamoeba histolytica* and are identified using conventional diagnostic methods, consist 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 infected 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 the Entamoeba with cells of the intestine epithelium, granulocytes, macrophages and fungi.
Sensitive immunological test procedures such as this rapid assay 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.

3. Test principle

This rapid assay is a single-step, immunochromatographic lateral-flow test where antibodies which are specifically directed against Entamoeba are attached to red latex particles. Other specific antibodies against the pathogen are firmly bound to the membrane. The stool sample is first suspended in the extraction buffer and then precipitated. An aliquot portion of the clear supernatant of the sample is placed on the test field. The sample with the coloured latex particles, to which the antigen is attached if present if the test is positive, then passes through the membrane and is bonded to the specific catch band.

4. Reagents provided

There are enough reagents in the pack for 20 determinations.

Cassette	20 det.	20 individually packed test cassettes
Buffer	26 ml	Extraction buffer, ready for use; contains 0.1 % sodium azide
Pipet	25 pieces	Bag containing 25 disposable pipet

Dangerous substances are indicated according to labelling obligations. For more details, refer to the Safety Data Sheets (SDS www.r-biopharm.com).

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. Likewise, the usability of the cassettes cannot be guaranteed once the external packaging of the individual cassette has been damaged.

6. Reagents required but not provided

6.1 Necessary reagents

No additional reagents are needed to perform this test.

6.2 Necessary laboratory equipment

The following equipment is needed to perform this test:

Equipment
Test tubes for stool suspension
Vortex mixer (optional)
Micropipet (200 µl - 1000 µl)
Waste container containing 0.5 % sodium hypochlorite solution

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for carrying out this test. Do not pipette samples or reagents by mouth. Avoid contact with broken skin and mucous membranes. Wear personal protective equipment (suitable gloves, apron, protective glasses) when handling reagents and samples and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are being processed.
For more details, refer to the Safety Data Sheets (SDS) at www.r-biopharm.com.

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

The reagents contain sodium azide as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

All reagents and materials that come into contact with potentially infectious specimens must be treated with suitable disinfectants (e.g., sodium hypochlorite) or autoclaved at 121 °C for at least one hour.

8. Collection and storage of specimens

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 (Tab. 1). 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.

Tab. 1: Specimen storage

Undiluted stool specimens	
2 - 8 °C	≤ - 20 °C
≤ 3 days	> 3 days

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 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 as this may cause reagent contamination.

9.2 Preparing the specimens

Place 1 ml Extraction Buffer Buffer in a labelled test tube. With the **liquid** stool sample, pipet 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 (size of a small pea) 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 pipet 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.

9.3 Specimen testing

When removed from the external packing, first lay the test cassette Cassette on a level mat. After this, pipette 200 µl (micropipet) or 4 drops (disposable pipet) Pipet 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 **5 minutes**.

10. Quality control – indication of instability or deterioration of reagents

The test must only be evaluated if the test cassette is intact **before** the sample suspension is pipetted in and no colour changes or bands are visible on the membrane. Furthermore, at least the **blue** 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 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 the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

A maximum of two bands should appear in the following order, as seen from the sample-absorption site: one red test band and one blue control band. **If the blue control band is missing, the test is invalid and cannot be evaluated!**

The following interpretations are possible:

- **Entamoeba positive**: the **red** and **blue** bands are visible.
- **Entamoeba negative:** only the **blue** band is visible.
- **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 5 minutes or later are also without any diagnostic value and must not be used for evaluation.

12. Limitations of the method

RIDA®QUICK Entamoeba detects antigens of *Entamoeba histolytica* sensu lato in stool samples. The test cannot be used to derive a relationship between the intensity of the specific visible band 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 *Entamoeba histolytica* infection. 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 pathogen 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 repeat the test with a smaller stool quantity or dilute the suspension already prepared further (clear supernatant after sedimentation) in order to clarify whether the pathogen being looked for is in the sample and has been masked by too much stool matrix.

13. Performance characteristics

13.1 Clinical comparison study

The sensitivity (**84.8 %)** and specificity (**87.4 %)** of the Entamoeba rapid assay strips agree with the values of the specific Entamoeba test band in the corresponding parasite combi rapid assay (N1722 RIDA®QUICK Cryptosporidium/ Giardia/ Entamoeba and N1723 RIDA®QUICK Cryptosporidium/Giardia/Entamoeba).

13.2 Cross-reactivity

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

Entamoeba coli, *Blastocystis hominis*, *Chilomastix mesnili*, *Endolimax nana*, *Entamoeba nana*, *Entamoeba hartmannii*, *Hymenolepsis nana*, *Isospora belli*, *Isospora felis*, *Jodamoeba bütschlii*

14. Version history

Version number	Chapter and designation
2010-08-10	Previous version
2019-07-10	General revision 4. Reagents provided 7. Warnings and precautions for the users 8. Collection and storage of specimens 9.2 Preparing the specimens

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Cassette	Test cassette
Buffer	Extraction buffer
Pipet	Disposable pipet

16. References

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