RIDA®QUICK Entamoeba

Article no: N1702
1. Intended use

For \textit{in vitro} diagnostic use. RIDA\textsuperscript{®}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 \textit{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, \textit{Entamoeba histolytica sensu stricto} and the non-pathogenic species (according to current knowledge), \textit{Entamoeba dispar}. Roughly 90\% of people infected with \textit{Entamoeba} infections have \textit{E. dispar}. The approximately 40-50 million cases of amoebic colitis or hepatic abscess every year which result in 80,000 deaths are caused by \textit{E. histolytica}.

The life cycle of the \textit{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. The test strip is dipped in the clear supernatant of the sample. During this time, the sample containing the coloured latex particles, to which the antigen is attached itself if present if the test is positive, passes through the membrane and is bound to the specific band.

4. Reagents provided

There are enough reagents in the pack for 25 determinations.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip</td>
<td>25 det.</td>
<td>Tube with 25 test strips</td>
</tr>
<tr>
<td>Diluent</td>
<td>26 ml</td>
<td>Extraction buffer, ready for use; contains 0.1 % sodium azide</td>
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<tr>
<td>Pipet</td>
<td>25 pieces</td>
<td>Bag containing 25 disposable pipettes</td>
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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. Additional necessary reagents – and necessary accessories

- 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 as this may cause reagent contamination.

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 (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 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 this, transfer between 200 µl and 500 µl 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 5 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 prepared sample suspension. 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 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 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 to 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
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; Hymenolepis nana; Isospora belli; Isospora felis; Jodamoeba bütschlii

References