RIDA®QUICK Cryptosporidium

Article no: N1203

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

1. Intended use

For *in vitro* diagnostic use. The RIDA®QUICK Cryptosporidium is a quick immunochromatographic test for the qualitative determination of Cryptosporidium parvum in stool samples.

2. Summary and explanation of the test

Cryptosporidium parvum is a parasite which is far more common in animals and occurs as an important pathogenic organism in domestic animals and in calves in particular. However, infestations in humans are observed in many countries now more frequently than was assumed earlier. In tropical developing countries, the parasite is often endemic and causes diarrhoea epidemics among children. In immunocompetent patients, the illness 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 diarrhoeas persist and are very severe. The infestation can be transmitted from animal to humans via contaminated water and from human to human. Members of communal facilities, children in kindergartens and 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 required the services of experienced personnel. An important alternative method to microscopy for the detection of Cryptosporidium parvum is the quick immunochromatographic test described in the following which, because it uses monoclonal antibodies, is equivalent to the microscopic 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 quick test is a single-step, immunochromatographic lateral-flow test where antibodies which are specifically directed against Cryptosporidia 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 strip. The sample then passes, with the coloured latex particles to which antigen are attached if the test is positive, 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
Diluent	26 ml	Extraction buffer, ready for use; contains 0.1 % sodium azide
Pipet	25 ea..	Bag with 25 disposable pipettes

5. Storage instructions

The pack can be stored at 2 – 30 ℃ 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. Materials required but not provided

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

7. Precautions for the 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 must be strictly adhered to.

The sample dilution buffer contains sodium azide as a preservative. This substance 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 or test reagents are being used.

All reagents and materials which come into contact with potentially infectious samples must be treated exactly like the samples themselves with suitable disinfectants (e.g. sodium hypochlorite) or autoclaved for at least one hour at 121 °C.

8. Specimen 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. Multiple freezing and thawing of the sample must be avoided.

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

Before using the samples, the extraction buffer and the test cassettes must be brought to room temperature (20 – 25 °C). 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 samples

Place 1 ml Extraction Buffer Diluent in the test tubes indicated. With the **liquid** stool sample, pipette 100 µl of the sample with an disposable pipette Pipet (up to just above the second thickening) and suspend it in the buffer placed in the tube beforehand. With **solid** stool samples, suspend 50 mg in the buffer. The sample must then be well homogenized. 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.

9.3. Testing the sample

When removed from the external packing, first lay the test cassette Cassette on a level mat. After this, pipette 200 µl of the clear supernatant of the stool suspension with a micropipette or 4 drops with a disposable pipette Pipet into the round opening of the test cassette. Makes 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 – indications of reagent expiry

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. In addition to this, at least the 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

After this, if the control band is still not visible after repeating the test with a new test cassette, please contact the manufacturer.

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:

- **Cryptosporidia positive:** the **red** and **blue** bands are visible.
- **Cryptosporidium 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, changes in band colour which only appear after 10 minutes or later are also without any diagnostic value and must not be used for evaluation.

12. Limitations of the method

The RIDA®QUICK Cryptosporidium detects antigens of Cryptosporidium parvum 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 Cryptosporidia infestation. This can be caused by intermittent excretion of the pathogen or by the quantity of antigens in the sample being too small. If the patient is anaemic or is suspected as being infested 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 precipitation), 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

In a routine laboratory, a comparative investigation was carried out between the RIDA®QUICK Cryptosporidium method and the established microscopy method on both frozen and fresh stool samples using 55 stool samples in total (15 Cryptosporidium positive and 40 negative stool samples). The results are listed in Table 1.

Table 1: Comparison of the RIDA®QUICK Cryptosporidium with microscopy

		RIDA®QUICK Cryptosporidium	
		+	**-**
Microscopy	**+**	14	1
	-	0	40

Sensitivity: 93.8 % **pos. prognosis value: 100.0 %**
Specificity: 100.0 % **neg. prognosis value : 97.5 %**

13.2. Cross reactivity

None that led to a cross reaction with the following specified intestine parasites in the RIDA®QUICK Cryptosporidium test:

Entamoeba coli
Blastocystis hominis
Jodamoeba buetschlii
Chilomastix mesnili
Endolimax nana
Eggs of Taenia spp.
Giardia lamblia

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