RIDA®QUICK

Rotavirus/Adenovirus Combi

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1. Intended use

For *in vitro* diagnostic use. The RIDA®QUICK Rotavirus/Adenovirus Combi test is a quick immunochromatographic test for the qualitative determination of rotaviruses and/or adenoviruses in stool samples.

2. Summary and explanation of the test

Adenoviruses were discovered in 1953 by Rowe and his coworkers in adenoid tissue. Their aetiological significance in acute respiratory disorders was then quickly recognised. Adenoviruses can cause acute pharyngitis, pharyngoconjunctival fever, bronchitis and pneumonias. About 5 % of the acute respiratory diseases in children under 5 years of age are attributed to adenoviruses. About 10 % of pneumonias in infancy are caused by adenoviruses. The importance of adenoviruses in gastrointestinal disorders was in doubt for a long time. Since then, however, it has been found that some adenovirus types which can only be cultured in cell cultures with difficulty cause gastrointestinal disorders. In the case of children with acute gastroenteritis, adenoviruses were found in 4 – 14 % of the stool samples examined. After rotaviruses, they are therefore the second most frequent cause of these symptoms and signs in infancy. Epidemic diseases caused by enteric adenoviruses have also been described. As with rotaviruses, a precise diagnosis of the pathogen is important in order to avoid hospital-borne infections. Rotaviruses are the most important pathogens in non-bacterial gastroenteritis in children from 6 months to 3 years old. They have also been shown to be the cause of illness in older children and adults. In risk groups, i.e. children and old or immune-suppressed patients, they can lead to death.

Rotavirus infections occur frequently during the winter months. Endemic diseases and epidemic diseases affecting some thousands of patients have also been described. In the case of hospitalised children with acute enteritis, up to 50 % of the samples examined are rotavirus positive. Rotaviruses transferred via the faecal-oral route are eliminated in large quantities into the intestine, so that hospital-borne infections from rotaviruses are regarded very seriously, particularly in baby stations and paediatric clinics, and are difficult to control. Early and reliable detection so that rotaviruses can be recognised and further infections avoided is therefore very important.

3. Test principle

This quick test is a single-step immunochromatographic lateral-flow test, where monoclonal antibodies which are directed against both viruses attach themselves to red (rotavirus-specific) or blue (adenovirus-specific) latex particles. Other specific antibodies against the two pathogens 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 antigens are attached if the test is positive, through the membrane and is bonded to the specific catch band. A blue and/or red band appears, depending on the antigens present in the sample.

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 pieces	Bag with 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. 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 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 lead to 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 a 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 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.

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. 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 – 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 green 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 or your local R-Biopharm distributor.

11. Evaluation and interpretation

A maximum of three bands should appear in the following order, as seen from the sample-absorption site: one blue (T_1 = Test band 1), one red (T_2 = Test band 2) and one green (C = Control band) band. **If the green control band is missing, the test is invalid and cannot be evaluated!**

The following interpretations are possible:

- **Adenovirus positive : blue** and **green** bands are visible.
- **Rotavirus positive : red** and **green** bands are visible.
- **Adenovirus and rotavirus positive : blue, red** and **green** bands are visible.
- **Negative:** only the **green** 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 Rotavirus/Adenovirus Combi test determines antigens of rotaviruses and/or adenoviruses 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 rule out rotavirus or adenovirus infection. 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 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

The sensitivity and specificity of this test were tested on the basis of clinical samples in comparison with a commercial Elisa. The results are listed in the following table.

Adenovirus		
	RIDA®QUICK	
Elisa	**+**	**-**
+	**9**	**1**
-	**0**	**189**

Sensitivity: **90 %**
Specificity: **100 %**
Pos. prognosis value : **100 %**
Neg. prognosis value : **99.5 %**

Rotavirus		
	RIDA®QUICK	
Elisa	**+**	**-**
+	**105**	**0**
-	**1**	**95**

Sensitivity : **100 %**
Specificity : **99 %**
Pos. prognosis value: **99.1 %**
Neg. prognosis value : **100 %**

The RIDA®QUICK Rotavirus/Adenovirus Combi rapid assay was also compared with another commercial combi rapid assay against PCR methods in a respective study. The specimens which were tested came from a multicentric study with children under four years old who were being treated for symptoms of gastroenteritis in hospital or as outpatients.

The results are listed in Table 2.

		RIDA®QUICK Rotavirus/Adenovirus Combi				**Coris Bioconcept Combi-Stick**			
		Rotavirus		**Adenovirus**		**Rotavirus**		**Adenovirus**	
		+	**-**	**+**	**-**	**+**	**-**	**+**	**-**
PCR	**+**	**45**	**1**	**32**	**12**	**44**	**2**	**23**	**21**
	-	**3**	**51**	**1**	**55**	**1**	**53**	**0**	**56**
Sensitivity		**97.8 %**		**72.7 %**		**95.7 %**		**52.3 %**	
Specificity		**94.4 %**		**98.2 %**		**98.1 %**		**100.0 %**	
PPV		**93.8 %**		**97.0 %**		**97.8 %**		**100.0 %**	
NPV		**98.1 %**		**80.9 %**		**96.4 %**		**72.7 %**	
Accuracy		**96.0 %**		**87.0 %**		**97.0 %**		**79.0 %**	

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