RIDA®QUICK Verotoxin/O157 Combi

Article no: N2203

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1. Intended use

For *in vitro* diagnostic use. The RIDA®QUICK Verotoxin/O157 Combi Test is an immuno-chromatographic rapid assay for the qualitative determination of verotoxins (syn. shigatoxins) and/or the E.coli Serovar O157 from a stool enrichment in mTSB broth.

2. Summary and explanation of the test

Shigatoxins (Stx) are the most important virulence factors of enterohaemorraghic Escherichia coli (EHEC). They cause diarrhoeas and the life-threatening haemolytic uraemic syndrome (HUS), which is one of the main causes of acute kidney failure in childhood. Serogroup O157 is involved in approx. 70-90% of all EHEC infections. A rapid determination of this pathogen is essential for deciding on a suitable therapy. The RIDA®QUICK Verotoxin/O157 Combi Test detects simultaneously the shigatoxins and Serogroup O157 in the supernatant of a stool enrichment in mTSB broth within just one day.

3. Test principle

This rapid assay is a single-step immunochromatographic lateral-flow test, where specific antibodies which are directed towards both target antigens are attached to red (shigatoxin-specific) or green (O157-specific) latex particles. Other specific antibodies against the two antigens are firmly attached to the membrane. The stool sample to be examined is first enriched in an enrichment broth (mTSB + Mitomycin C) 37°C for approx. 18-24 h in order to propagate the pathogen and form the shigatoxin. After centrifuging the broth, an aliquot portion of the supernatant is diluted 1:2 with the test-specific sample buffer and the diluted mixture used in the test. When placing the diluted sample made in this way in the round opening of the test cassette, specific antibodies which are bound to coloured latex particles attach themselves to the antigens in the sample and flow via the membrane to the specific collection bands where they are again bound specifically. A green and/or red band appears, depending on the antigens present in the sample. The blue control band must also always appear to confirm that the test is valid.

4. Reagents provided

There are enough reagents in the pack for 20 determinations.

Cassette	20 det.	20 individually packed test cassettes
Diluent	18 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
- Enrichment broth (mTSB + Mitomycin C ; e.g. RIDA® Anreicherungsbouillon : Z1003)
- Centrifuge

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

If the stool samples to be tested for shigatoxin or E.coli O157 are not used fresh, they can be stored in the enrichment broth as follows before use:
– up to 24 hours at room temperature or at 2 – 8°C
– up to 72 hours at 2 – 8°C
Freezing the samples is not recommended since this will damage the EHEC bacteria and reduce their ability to multiply or even stop them from multiplying at all in the enrichment culture.
Stool samples must be collected in clean containers without any additives and stored at 2 – 8°C before beginning the test.
If rectal swabs have to be used, make sure that sufficient stool material (approx. 100 mg) is collected to carry out the test.

During contact tracing, stool samples should also be taken from clinically inconspicuous contacts in order to identify asymptomatic carriers.

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.

9.2. Preparing the samples

First, place 100 µl liquid stool or an equivalent quantity (50-100 mg) of solid stool sample in 4 ml mTSB with Mitomycin C (e.g. RIDA® Anreicherungsbouillon, Article no: Z1003) and incubate it on a shaker (approx. 120-160 rpm) with an adequate supply of oxygen at 37°C for 18 to 24 hours maximum. A horizontal shaker and rotary mixer are both suitable for use as a shaker. After incubation, centrifuge the entire sample at 2500 g for 5 min. Dilute the clear supernatant 1:2 with specific extraction buffer and transfer the diluted mixture to a clean test tube or uncoated microtitre well.

9.3. Testing the sample

When removed from the external packing, first lay the test cassette Casette on a level mat. After this, use a micropipette to pipette 200 µl or a disposable pipette Pipet to add 4 drops 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 1**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 into it and no colour changes or bands are visible on the membrane. In addition to this, 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 three bands should appear in the following order, as seen from the sample-absorption site: one green (T_1 = Test band 1), one red (T_2 = Test band 2) and one blue (C = Control band) band. **If the blue control band is missing, the test is invalid and cannot be evaluated!**

The following interpretations are possible:

- **O157 positive : green** and **blue** bands are visible.
- **Shigatoxin positive : red** and **blue** bands are visible.
- **O157 and Shigatoxin positive : green, red** and **blue** bands are visible.
- **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 15 minutes are also without any diagnostic value and must not be used for evaluation.

12. Limitations of the method

The RIDA®QUICK Verotoxin/O157 Combi test determines antigens of O157 / or Shigatoxins in enriched 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 rule out possible EHEC infection or the presence of shigatoxins. This can be because the quantity of antigens in the sample is 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.

13. Performance characteristics

13.1. Test quality

The test validation was carried out on a total of 159 samples in the control laboratory for HUS in the Institute of Hygiene at Münster University. These included 51 EHEC reference strains which represented a broad spectrum of 38 different serotypes. All the other shigotoxin types which are pathogenic to humans up to the subtype Stx 2g which is pathogenic to animals were represented. 96 stool samples and 8 isolates from these samples were also examined as well as 4 isolates from animals. The results were compared with Stx- and O157-specific PCR methods from the Institute. A cytotoxicity test with verocells was also used to verify the Stx production The results are listed in Table 1.

Table 1:
Comparison of the RIDA®QUICK Verotoxin/O157 Combi rapid assay with specific PCR methods for the determination of Stx coded genes as well as the sfpA genes specific for Serotype O157.

		RIDA®QUICK Verotoxin/O157 Combi			
		Stx 1 / Stx 2		O157	
		+	-	+	-
PCR	+	68	12	20	2
	-	1	78	2	135

Sensitivity:	85.0 %	90.9 %
Specificity:	98.7 %	98.5 %
pos. predictive value :	98.6 %	90.9 %
neg. predictive value :	86.7 %	98.5 %
Accuracy	91.8 %	97.5 %

13.2. Analytical sensitivity

Both toxins were serial diluted to determine the detection limit for both shigatoxins and used in the RIDA®QUICK Verotoxin/O157 Combi Test. The results are shown in Table 2.

Table 2: Detection limit for Stx 1 and Stx 2

Stx 1 pure ng/ml	RIDA®QUICK Stx / O157	Stx 2 pure ng/ml	RIDA®QUICK Stx / O157
50	**++++**	**50**	**++++**
40	**++++**	**40**	**++++**
30	**+++**	**30**	**++++**
20	**+++**	**20**	**++++**
10	**++**	**10**	**++++**
8	**++**	**8**	**+++**
6	**++**	**5**	**+++**
4	**+**	**4**	**++**
2 *	**(+)**	**2**	**++**
1	**-**	**1**	**+**
0.5	**-**	**0.5 ***	**(+)**
0.1	**-**	**0.1**	**-**

* Detection limit

13.3 Specificity

The RIDA®QUICK Verotoxin/O157 Combi Test did not show any cross-reactivity with other E-coli serotypes or other enterobacteriaceae except for Group N salmonellae which also have the O157 antigen on their surface. This surface antigen is occasionally found with a few strains of the species Citrobacter so that if there is a reaction with the O157 band alone, identification after culturing is advisable. On the other hand, O157 isolates which have lost the ability to produce shigatoxins are very rare (phage loss).

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