RIDASCREEN® Verotoxin

Article no.: C2201

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**.** RIDASCREEN® Verotoxin is an enzyme immunoassay for the qualitative detection of verotoxins (syn. shiga toxins) from a stool enrichment in mTSB broth.

2. Summary and explanation of the test

Enterobacteriaceae of the *Escherichia coli* (*E. coli*) type are constituents of a healthy intestinal flora in humans, but also in many farm animals. Therefore they are also considered an indicator of fecal contamination of bodies of water and foods. These are gram-negative, optionally anaerobic rod-shaped bacteria that can move by peritrichous flagellation. Based on O-antigens (LPS of the outer membrane) and H-antigens (flagellar antigens), *E. coli* can be divided into various serovars. *E. coli* must be considered optionally pathogenic due to various pathogenicity factors which are often plasmid encoded or transmitted by phages. Since 1977, *E. coli* have been known that can produce two cytotoxins, verotoxin 1 and verotoxin 2. The genes for these verotoxins (VT) are located on the chromosome in the region of a prophage. *E. coli* have been isolated that included both genes or just one of the two. Because of the similarity of the verotoxins to the shiga toxin from *Shigella dysenteriae*, they are also called shiga-like toxins I and II (SLT-I and -II). A part of these verotoxigenic *E. coli* (VTEC) can cause severe diarrheas by forming further pathogenicity factors. The clinical symptoms caused by EHEC range from slight diarrheas to severe gastroenteritides to hemorrhagic colitis, which occurs in approx. 10 to 20% of infections. A life-threatening post-infectious complication can occur in 5 to 10% of infections, particularly in infants and young children, but also in old or immunocompromised patients: hemolytic-uremic syndrome (HUS) or thrombotic-thrombocytopenic purpura (TTP). Mortality of HUS and TTP is high, particularly in childhood (approx. 10 to 15%). They can lead to acute kidney failure with temporary dialysis dependency or an irreversible loss of the kidney function and resulting permanent dialysis dependency. Some of the former methods to diagnose an EHEC infection have proven insufficient. Since only approx. 1% of the *E. coli* that do not belong to the EHEC group can form an enterohemolysin, detection of enterohemolytic coliform bacteria on blood agar plates is certainly an important indication of EHEC. But there are also EHEC that were isolated from HUS patients and do not express enterohemolysin. It also turned out that the O157 type found first is only responsible for 70 to 80% of the HUS cases in Germany. Other serovars are even predominant in pure gastroenteritides. Sole detection of the O157 serovar is therefore insufficient. The recommended diagnostic procedure of today is preconcentration of the pathogen over night in mTSB medium (with 50ng/ml mitomycin C added) and subsequent isolation. At the same time, an attempt should be made to detect verotoxin as the safest pathogenicity factor from the culture supernatant. This can be done immunologically by an ELISA or by demonstrating the cytopathic effect on a vero cell culture. Unlike the cytotoxicity test for vero cells, the ELISA technique as offered by the RIDASCREEN® Verotoxin EIA is a simple method that can be performed in any laboratory. Preconcentration of the germ is required for both methods, since germ excretion and the toxin content in the stool can be very low. Excretion can also have stopped completely, particularly in HUS and TTP patients. Direct detection of the tox-

in in the stool sample without preceding enrichment can be attempted to get a fast result in an individual case. Since this procedure lacks sensitivity, a negative result should only be viewed as a preliminary indication. One should wait for the result of the enrichment. An antibacterial chemotherapy is generally not indicated because it would prolong bacteria excretion and can even stimulate toxin formation.

3. Test principle

The RIDASCREEN® Verotoxin Test uses specific antibodies in a sandwich-type method. Monoclonal antibodies to verotoxins 1 and 2 are coated to the well surface of the microwell plate. A suspension of the stool sample to be tested or the supernatant of an overnight enrichment of the stool sample in mTSB broth and the controls are pipetted together with the biotinylated anti-verotoxin antibodies (Conjugate 1) into the respective well of the microwell plate at room temperature (20–25°C).After a washing stage, poly-streptavidin-peroxidase conjugate (Conjugate 2) is added and incubated is carried out again at room temperature (20–25°C). If verotoxins are present in the sample, a sandwich complex will form which consists of immobilized antibodies, verotoxins, and the anti-verotoxin antibodies biotinylated with the poly-streptavidin-peroxidase complex. A further washing stage removes the unattached poly-streptavidin-peroxidase conjugate. In positive samples, the addition of a substrate changes the bound enzyme from a colorless solution to a blue solution. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of verotoxins found in the sample.

4. Reagents provided

The reagents in the kit are sufficient for 96 assays.

Plate	96 det..	Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with monoclonal antibodies to verotoxin 1 and 2
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution, ready to use, blue colored
Wash	100 ml	Wash buffer, phosphate buffered NaCl solution (concentrated 10-fold);contains 0.1% thimerosal
Control | +	2 ml	Positive control; inactivated verotoxin culture; ready for use
Control | -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate | 1	13 ml	Biotin-conjugated antibodies to verotoxin 1 and 2 in stabilized protein solution; ready to use; purple color
Conjugate | 2	13 ml	Poly-streptavidin-peroxidase conjugate in stabilized protein solution; ready for use; orange color
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use

Stop	12 ml	Stop reagent; 1 N sulfuric acid; ready for use

5. Reagents and their storage

All reagents must be stored at 2–8°C and can be used until the date printed on the label. Providing the diluted wash buffer is stored at 2–8°C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid. The aluminum bag must be opened with scissors in such a way that the clip seal is not separated. Any microtiter strips which are not required must be returned to the aluminum bag and immediately stored at 2–8°C. The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Additionl necessary reagents – and necessary equipment

6.1. Reagents

- Distilled or deionized water

6.2. Accessories

- RIDA® enrichment broth (Art. no. Z1000)
- Disposable pipettes (Art. no.: Z0001)
- Vortex mixer (optional, see 9.3.)
- Micropipette for 50 - 100 µl and 1 ml volumes
- Measuring cylinder (1000 ml)
- Stop clock
- Washing device for microwell plates or multichannel pipettes (300 µl)
- Photometer for microwell plates (450 nm and reference filter 620-650 nm)
- Filter paper (laboratory towels)
- Waste container with a 0.5 % hypochlorite solution

7. Precaution for users

For *in vitro* diagnostic only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always strictly adhere to the user instructions for this test.

The kit includes a positive control that contains the inactivated verotoxin. Like the patient samples, it must be treated as potentially infectious material and handled in accordance with national safety regulations.

Samples or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be avoided. 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

The wash buffer contains 0.1 % thimerosal as preservative. This substance must not be allowed to come into contact with skin or mucous membranes.

Urea peroxide can cause chemical burns. Handle with care.

The Stop reagent contains 1 N sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the reagent, rinse it off with water.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121°C for at least 1 hour. CAUTION: To prevent the formation of toxic gases, any liquid waste containing stop reagent must be neutralized before adding to hypochlorite solution.

8. Specimen collection and storage

If not used when fresh, the stool samples to be tested for verotoxins can be stored as follows:

- up to 24 hours at room temperature or at 2 - 8°C
- up to 72 hours at 2–8°C

We do not recommend freezing the samples because the EHEC bacteria may suffer and not be able to multiply as well, or multiply at all, in the enrichment culture.
Stool samples must be collected in clean containers without additives and stored at 2 - 8°C before the beginning of the test.
Stool samples and rectal smears should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® Verotoxin Test.
If rectal smears are used, make sure that the volume of stool material is sufficient (approx. 100 mg) for the test.
Contact tracing should include stool samples taken from contact persons who do not exhibit clinical symptoms, in order to identify asymptomatic carriers.

9. Test procedure

9.1. General information
All reagents and the microwell plate Plate must be brought to room temperature (20 - 25°C) before use. The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. After use, the microwell strips (placed in sealed bags) and the reagents must be stored again at 2–8°C. Once used, the microwell strips must not be re-used. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking. In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components. The test must not be carried out in direct sunlight. We recommend covering the microwell plate or sealing with plastic wrap to prevent evaporation losses.

9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate Wash with 9 parts distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37°C.

9.3. Preparing the samples

A direct toxin detection test from the stool sample can be performed to get an initial result. Since the toxin concentration in the stool may be low, a negative result should not be considered a negative EHEC result. Toxin detection should in general be based on the enrichment culture – even if the stool results are negative – to achieve greater sensitivity. In Germany, the current recommendation is enrichment over night in a shake culture at 37°C in mTSB medium with mitomycin C (50 ng/ml) added. Other enrichment media such as GN broth did not show negative influences (matrix effects) in the RIDASCREEN® Verotoxin ELISA; but they are not the enrichment medium recommended by professional associations.

9.3.1. Examination of fresh or frozen stool samples

Dilute the stool sample at a ratio of 1:11 (v/v) with verotoxin sample dilution buffer. Take up 1 ml liquid stool using a disposable pipette (item no.: Z0001) and mix it with 4 ml buffer in a labeled test tube. Pick up a respective volume of solid stool using a disposable inoculation loop. Homogenize the stool suspension by suction and ejection from a disposable pipette, or, alternatively, by mixing in a vortex mixer. Allow the batch to stand for a short time (10 min, clarifying by sedimentation), 100 µl of the supernatant of the suspension are used directly in the test. If the test procedure is carried out in an automated ELISA system, the supernatant must be particle-free. In this case, it is advisable to centrifuge the sample at 2500 G for 5 minutes.

Important: Due to its low sensitivity, detection from the unenriched stool sample can only be considered a fast pre-screening in cases of anamnestic urgency. A negative result is not valid. Instead, wait for the result from tests on the enriched sample.

9.3.2. Examination of samples from the enrichment culture

The procedure described here is compliant with the recommendations of RKI (Robert Koch Institute) and BfR (Federal Institute of Risk Assessment). Please comply with the testing instructions for the RIDA® enrichment broth (item no. Z1000). The supernatant of an enrichment culture (RIDA® enrichment broth) can be directly used in the test without further dilution after brief centrifuging.

Important: Make sure that the temperatures of the broth and the negative control (both should be adjusted to room temperature (20 - 25°C) at the beginning of the test) are equalized. Elevated broth temperatures can cause false positive OD values.

First, transfer 100 µl of liquid stool or an equivalent quantity (50 - 100 mg) of a solid stool sample into 4 ml mTSB with mitomycin C (e. g. RIDA® enrichment broth, item no. Z1000) and incubate under sufficient supply of oxygen (half turn of the screw cap) in inclined position at 37°C for

18 to a maximum of 24 hours. Centrifuge the entire sample after incubation for 5 min at 2,500 x g. 100 µl of the clear supernatant are used in the ELISA test.

Store the sediments and the original samples for later confirmation. Ideally, pick up the sediment with a swab and transfer it into a suitable transport medium (Amies, Stuart or Cary-Blair), then store it at 2–8°C until it is shipped.

9.4. First incubation

After inserting a sufficient number of wells in the strip holder, pipette 100 µL of the Control | + positive control, the Control | - negative control, the enrichment culture (or optionally the stool sample suspension) into the wells. Then add 100 µl of the Conjugate | 1 biotin-conjugated antibody and blend (by tapping lightly on the side of the plate), then incubate for 60 minutes at room temperature (20 - 25 °C).

9.5. Washing

Careful washing is important in order to achieve the correct results and should therefore be carried out strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container for disposal in accordance with official regulations. After this, tap out the plate onto absorbent paper in order to remove the residual moisture. Then wash the plate five times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by tapping them out after each wash onto a part of the absorbent paper which is still dry and unused.

If a microplate washer or fully automated ELISA is used, ensure that the machine is correctly adjusted or request the settings from the manufacturer if necessary. Appliances delivered by R-Biopharm are already programmed with validated settings and work protocols. To avoid blocking the wash needles, only particle-free stool suspensions should be dispensed (see Item 9.3., Preparing the samples). Also ensure that all of the liquid is aspirated during each washing stage.

9.6. Second incubation

Use a pipette to fill 100 µl Conjugate | 2 poly-streptavidin peroxidase conjugate into the wells, then incubate for 30 minutes at room temperature (20 - 25°C).

9.7. Washing

Wash as described in Item 9.5.

9.8. Third incubation

Fill all wells with 100 µl substrate Substrate. Then incubate the plate for 15 minutes in darkness at room temperature (20 - 25°C). Subsequently fill all wells with 50 µl stop reagent Stop in order to stop the reaction. After blending cautiously by tapping lightly on the side of the plate,

measures the extinction at 450 nm (optional: 450/ 620 nm). Adjust the zero point in the air, i.e. without the microwell plate.

Note:
High-positive patient samples may cause black-colored precipitates of the substrate.

10. Quality control – indications of reagent expiry

For quality control purposes, positive and negative controls must be used each time the test is carried out, to ensure that the test has been carried out correctly and that the reagents are stable. The test has been carried out correctly if the extinction rate (OD) for the negative control is less than 0.2 at 450 nm (less than 0.160 at 450/620 nm) and the measured value for the positive control is greater than 0.8 at 450 nm or at 450/620 nm. A value greater than 0.2 (0.160) for the negative control may indicate that washing was insufficient. Deviation from the required values, just like a turbid or blue coloring of the colorless substrate before it is filled into the wells, may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Assessment and interpretation

11.1. Calculating the cut-off

The evaluation of the test is based on the same cutoff calculation for both types of testing material (supernatant from the enrichment culture or native stool sample).

cut-off = extinction for the negative control + 0.150

11.2. Test result

Assessment of the sample is **positive** if the extinction rate is more than 10% higher than the calculated cut-off value.

Assessment of the sample is **marginal** and the test needs to be repeated if the extinction rate ranges from 10% less to 10% greater than the cut-off value. If the repeat examination with a fresh stool sample again falls within the gray zone, assessment of the sample is negative.

Samples which are more than 10% below the calculated cut-off must be considered **negative**.

12. Limitations of the method

The RIDASCREEN® Verotoxin-Test detects the verotoxins 1 and 2 of verotoxigenic *E. coli* in stool samples or from enrichment cultures. It is not possible to associate the determined level of extinction to the occurrence or severity of clinical symptoms. **The results obtained must always be interpreted in combination with the clinical signs and symptoms. A positive toxin result and simultaneous identification of the germ is considered the safest method of detecting EHEC.**

A **positive** result does not rule out the presence of other infectious pathogens.

A **negative** result does not rule out the possibility of an infection with verotoxigenic *E. coli*. Such a result may be due to intermittent excretion of the pathogen, or the amount of antigen in the sample may be too small. If the patient history supports a suspicion of an infection with verotoxigenic *E. coli*, the examination should be repeated with another stool sample.

Some EHEC strains produce only small quantities of verotoxin. Because of the often low toxin content of a stool sample, direct toxin detection from stool as the sole method is not sufficiently sensitive.
A positive result from stool gives a fast indication of a potential EHEC infection. But an EHEC infection cannot be ruled out if the result is negative.

A **marginal** result may be due to non-homogeneous distribution of bacteria in the stool sample. In this case, examination should either be repeated with a second suspension from the same sample or another stool sample should be requested.

If the toxin detection is positive, it should be followed up by isolating *E. coli*. A connection between the detected toxin and the clinical symptoms can only be established through identification and characterization of the pathogen.

The toxin detection from an enrichment culture can be negative although an EHEC infection is present. Enrichment can be suboptimal depending on the culture conditions. Despite the successful cultivation of E. coli, the toxin content in the culture medium can be so low that a test for it turns out negative. The reason can be the low percentage of EHEC in the physiological *E. coli* flora (sometimes just one EHEC per 200 - 300 *E. coli*), or toxin production and excretion is suspended under specific growth conditions. It is therefore possible that toxin detection directly from the stool is (weakly) positive while detection from the culture is
negative.

Because of the highly sensitive setting of the RIDASCREEN® Verotoxin ELISA, a marginal or weakly positive result when examining native stool can also be caused by unspecific matrix effects. Therefore thorough washing is imperative for the direct examination of stool.

When post-infectious symptoms like HUS or TTP develop, the amount of pathogens in the stool can have dropped down to a point at which the germ can no longer be isolated. In this case, the verotoxin ELISA will be negative as well. It is therefore important that stool samples are examined as early as possible during the enteritic phase.

Otherwise please refer to the multi-stage plan of EHEC diagnostics recommended by the National Reference Center of Robert Koch Institute (Ref. 15).

13. Performance characteristics

13.1. Test quality

A validation study with RIDASCREEN® Verotoxin ELISA examined 94 samples. The samples were stool samples enriched in mTSB broth from a German routine laboratory which were frozen and stored for later use after the routine examination for the validation of the RIDASCREEN® Verotoxin ELISA at their laboratory. After thawing, the samples underwent comparative examination with RIDASCREEN® Verotoxin ELISA and two further commercial ELISAs. The results of the study are summarized in Table 1.

Table 1: Correlation between the RIDASCREEN® Verotoxin ELISA and two further commercial ELISAs

		ELISA 1		ELISA 2	
		Positive	Negative	Positive	Negative
RIDASCREEN® Verotoxin	Positive	35	4	34	5
	Negative	0	55	0	55

Positive match:	94.6 %	93.2 %
Negative match:	96.5 %	95.7 %

13.2. Cross reactivity

Various pathogenic organisms were investigated with RIDASCREEN® Verotoxin ELISA and showed no cross reactivity except for *S. aureus*. These studies were conducted with bacterial suspensions shown to have concentrations of 10^6 to 10^9 organisms per ml. Virus culture supernatants are listed accordingly.

S. aureus was introduced at a concentration of ~ 6.3 x 10^7 CFU/ml into the test and showed weak positive results in 2 out of 3 replicates. The results of that study are listed in Table 2.

Table 2: Cross reactivity with pathogenic microorganisms

Organism	Origin	Mean value [OD 450/620]
Adenovirus	Cell culture supernatant	-0.006
Aeromonas hydrophila	Culture	0.043
Astrovirus	Cell culture supernatant	0.000
Bacillus cereus	Culture	-0.001
Bacteroides fragilis	Culture	0.002
Campylobacter coli	Culture	0.021
Campylobacter jejuni	Culture	0.028
Candida albicans	Culture	0.006

Citrobacter freundii	Culture	-0.005
Clostridium difficile	Culture	-0.004
Clostridium perfringens	Culture	-0.001
Clostridium sordellii	Culture	0.056
Cryptosporidium muris	Culture	-0.001
Cryptosporidium parvum	Culture	-0.001
Entamoeba histolytica	Culture	0.003
Enterobacter cloacae	Culture	0.003
Enterococcus faecalis	Culture	0.005
Escherichia coli	Culture	-0.007
Escherichia coli	Culture	-0.001
Escherichia coli	Culture	-0.006
Escherichia coli	Culture	-0.005
Escherichia coli	Culture	-0.002
Escherichia coli	Culture	-0.006
Escherichia coli	Culture	-0.002
Escherichia coli	Culture	-0.002
Escherichia coli	Culture	-0.006
Escherichia coli (O26:H-)	Culture	-0.006
Escherichia coli (O6)	Culture	-0.002
Giardia lamblia	Stool	-0.001
Klebsiella oxytoca	Culture	0.003
Proteus vulgaris	Culture	0.004
Pseudomonas aeruginosa	Culture	-0.004
Rotavirus	Cell culture supernatant	0.001
Salmonella enteritidis	Culture	-0.005
Salmonella typhimurium	Culture	0.001
Serratia liquefaciens	Culture	0.002
Shigella dysenteriae	Culture	0.004
Shigella flexneri	Culture	-0.002
Shigella sonnei	Culture	-0.005
Staphylococcus aureus	Culture	0.126
Staphylococcus epidermidis	Culture	-0.003
Vibrio parahaemolyticus	Culture	-0.003
Yersinia enterocolitica	Culture	-0.005

13.3. Precision

The reproducibility of the RIDASCREEN® Verotoxin ELISA was tested with six references representing the complete measurement range from weak to high positive. To determine the intra-assay reproducibility, 40 replicates of these references were assayed. The mean values and the variation coefficients (VC) were determined for 3 lots. For the inter-assay reproducibility, references from 10 different working days were assayed in duplicates, with 2 runs per day. The measurements were determined in 3 lots by 2 technicians. The inter-lot reproducibility was determined for all 3 lots. The results are shown in Table 3.

Table 3: Results for the reproducibility/precision of RIDASCREEN® Verotoxin ELISA

Reference Mean value / VC		Intra-assay			Inter-assay			Inter-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1–3
1	MV	2.387	2.021	2.374	2.371	2.098	2.448	2.306
	VC (%)	5.01%	5.91%	7.61%	9.01%	7.56%	8.76%	11.58%
2	MV	1.497	1.402	1.753	1.706	1.518	1.779	1.668
	VC (%)	5.01%	5.21%	7.40%	15.59%	8.69%	10.20%	12.67%
3	MV	0.808	0.711	0.835	0.850	0.784	0.949	0.861
	VC (%)	8.60%	10.94%	9.32%	10.85%	10.08%	12.81%	14.85%
4	MV	0.310	0.280	0.379	0.386	0.371	0.452	0.403
	VC (%)	9.15%	9.39%	13.49%	15.33%	11.71%	14.98%	17.63%
5	MV	0.200	0.190	0.302	0.224	0.223	0.278	0.242
	VC (%)	12.23%	13.40%	7.94%	21.67%	17.17%	18.22%	22.74%
6	MV	-0.003	0.002	-0.002	0.010	0.004	0.012	0.009
	VC (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

13.4. Analytical sensitivity

To determine the analytical sensitivity of the RIDASCREEN® Verotoxin ELISA the limit of blank (LoB) was analyzed in 90 assays of negative samples (enrichment broth), and the limit of detection (LoD) was analyzed in 72 assays of the two toxins. The results of these measurements are shown in Table 4.

Table 4: Results for the analytical sensitivity of the RIDASCREEN® Verotoxin ELISA

	MV [OD 450/620]	pg/ml
LoB	0.022	-
LoD Verotoxin 1	-	12.5
LoD Verotoxin 2	-	25.0

13.5. Interfering substances

The following list of substances showed no effects on the test results when they were blended into the supernatants of verotoxine positive and verotoxine negative stool samples in the de-

scribed concentrations**:** Mucin (5% v/v), human blood (5% v/v), barium sulfate (18.5% v/v), loperamide (0.02% w/v), peptobismol (6.6% v/v), stearic/palmitic acid (40% v/v), metronidazole (3% w/v), diclofenac (0.1% w/v), cyclamate/saccharin mixture (1.3% v/v).

Mucin and barium sulfate also displayed a dose-dependent reduction of OD values if used further diluted from the concentrations mentioned above.

Appendix

Test specific symbols:

Plate	Microwell plate
Diluent | 1	Sample dilution buffer
Wash	Wash buffer
Control | +	Positive control
Control | -	Negative control
Conjugate | 1	Conjugate 1
Conjugate | 2	Conjugate 2
Substrate	Substrate
Stop	Stop reagent

Bibliography

1. Beutin, L. et al.: Zur Epidemiologie von Infektionen durch enterohämorrhagische E. coli (EHEC) in der Bundesrepublik Deutschland im Jahr 1993. Bundesgesundhbl. 10, 410 - 414 (1994)
2. Beutin, L.: Infektionen mit enterohämorrhagischen Escherichia coli (EHEC). Bundesgesundhbl. 11, 426 - 429 (1996)
3. Bockemühl, J. et al.: Zur aktuellen Bedeutung der enterohämorrhagischen Escherichia coli (EHEC) in Deutschland (1994-1995). Bundesgesundhbl. 8, 290 - 296 (1996)
4. Bockemühl, J. et al.: Infektionen des Menschen durch enterohämorrhagische Escherichia coli (EHEC) in Deutschland, 1996. Bundesgesundhbl. 6, 194 - 197 (1997)
5. Bülte, M.: Enterohämorrhagische E. coli-Stämme (EHEC) - Aktuell in der Bundesrepublik Deutschland? 1. Pathogenitätspotential von EHEC-Stämmen - Bedeutung als Lebensmittelinfektionserreger. (Literaturübersicht) Fleisch-wirtschaft 75, 1430 - 1432 (1995)
6. Bülte, M. et al.: Enterohämorrhagische E. coli (EHEC) - aktuelle Lebensmittelinfektionserreger auch in der Bundesrepublik Deutschland? 2. Nachweis von VTEC-Stämmen in Lebensmitteln tierischen Ursprungs Fleischwirtschaft 76, 88 - 91 (1996)
7. Donohue-Rolfe, A. et al.: Purification of Shiga Toxin and Shiga-Like Toxins I and II by Recptor Analog Affinity Chromatography with Immobilized P1 Glycoprotein and Production of Cross-Reactive Monoclonal Antibodies. Infection and Immunity, Dec., 3888 - 3893 (1989)
8. Karch, H. et al.: Clonal Structure and Pathogenicity of Shiga-Like Toxin-Producing, Sorbitol-Fermenting Escherichia coli 0157:H^-. Journal of Clinical Microbiology 5, 1.200 - 1.205 (1993)
9. Kuntze, U. et al.: Nachweis von verotoxinbildenden E. coli-Stämmen in Rohmilch und Rohmilchkäse. Archiv f. Lebensmittelhygiene 47, 141 - 144 (1996)
10. Ritchie, M. et al.: Comparison of a Direct Fecal Shiga-Like Toxin Assay and Sorbitol-McConkey Agar Culture for Laboratory Diagnosis of Enterohemorrhagic *Escherichia coli* Infection. J. of Clin. Microbiol., Feb., 461 - 464 (1992)
11. Strockbine, N. A. et al.: Characterization of Monoclonal Antibodies against Shiga-Like Toxin from *Escherichia coli*. Infection and Immunity, Dec., 695 - 700 (1985)
12. Takeda, Y. et al.: Vero Toxins (Shiga-Like Toxins) Produced by Enterohemorrhagic *Escherichia coli* (Verocytotoxin-Producing *E. coli*). Microbiol. Immunol., 37 (8), 591 - 599 (1993)
13. Walterspiel, J. N. et al.: Effect of Subinhibitory Concentrations of Antibiotics on Extracellular Shiga-like Toxin I. Infection 20, No. 1, 25 - 29 (1992)
14. Gerritzen, A. et al.: Flüssige Vorkultur von Stuhlproben verbessert den EHEC-Toxinnachweis mit ELISA und Zytotoxizitätstest. J. Lab. Med. 22 (2), 097 - 101 (1998)
15. Fruth, A. et al.: Zur Verbesserung der gegenwärtigen bakteriologischen Diagnostik von enterohemorrhagischen Escherichia coli (EHEC). Bundesgesundheitsbl.-Gesundheitsforsch.-Gesundheitsschutz 43, 310-317 (2000)