RIDASCREEN® Campylobacter

Article no.: C2401

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® Campylobacter is an enzyme immunoassay for the qualitative identification of *Campylobacter jejuni* and *Campylobacter coli* antigens in human stool samples and in cultures.

2. Summary and explanation of the test

Campylobacteriosis is only exceeded by salmonellosis as the most frequent cause of diarrhea in humans worldwide. The large increase in cases of enteritis caused by Campylobacter bacteria is exacerbated by their wide, cross-species prevalence among wild animals as well as domesticated animals used for work or as household pets (birds and mammals).

These bacteria enter the human food chain as commensal bacteria in the intestinal tracts of poultry in particular. But other vehicles for transport of the pathogens are foods such as milk and ground meat as well as drinking water. Large amounts of Campylobacter pathogens which are released to the environment by a multitude of hosts eventually infect humans by way of contaminated food. Other possible routes of transmission of Campylobacter enteritis are the direct contact with domesticated animals which are infected with campylobacteriosis and, particularly in children, the fecal-oral route. The infection dose of 500 microorganisms is relatively low. Of the roughly 15 known species of Campylobacter, *C. jejuni* and *C. coli* are the ones that cause gastroenteritis in humans. After an incubation period of 2 to 10 days, untreated individuals will excrete the infectious pathogens for up to four weeks in feces. Individuals with immune deficiency may continue to excrete the pathogens indefinitely. Many of the infections take an asymptomatic course, but those who become ill after the prodromal stage suffer fever, headache, myalgia, arthralgia, and fatigue in addition to diarrhea, abdominal cramps, and abdominal pain as the typical symptoms of enteritis. In consistency the diarrhea ranges from a mushy to massive, watery stool, sometimes also mixed with blood. Joint inflammation and the rare occurrence of Guillain-Barré syndrome are late sequelae of the disease.

Symptomatic treatment with fluid and electrolyte replacement is most common; antibiotic treatment is only applied in the severe cases of illness. Success in culturing these sensitive pathogens requires the freshest possible stool samples and cooled transportation over short distances. Modern techniques for identification of the antigens do not depend on those prerequisites, for example the RIDASCREEN® Campylobacter ELISA, which detects the specific Campylobacter antigen in a stool sample after the pathogens can no longer be cultured.

3. Test principle

The RIDASCREEN® Campylobacter Test employs monoclonal antibodies in a sandwich-type method. The well surface of the microwell plate is coated with monoclonal antibodies to *Campylobacter* antigens.

A pipette is used to place a suspension of the stool sample to be examined as well as control specimens into the well of the microwell plate together with biotinylated anti-*Campylobacter* antibodies (Conjugate 1) for incubation at room temperature (20 – 25 °C). After a wash step,

streptavidin poly-peroxidase conjugate (Conjugate 2) is added and it is incubated again at room temperature (20 – 25 °C). With the presence of *Campylobacter* antigens in a specimen, immobilized antibodies, antigens, and conjugated antibodies form a sandwich complex. Another wash step removes the unattached streptavidin poly-peroxidase conjugate. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. Addition of a stop reagent changes the color from blue to yellow. The extinction is proportional to the concentration of the *Campylobacter* antigen that is found in the specimen.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96	Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with specific monoclonal antibodies to the antigens of *Campylobacter jejuni* and *Campylobacter coli*
Diluent | 1	100 ml	Sample dilution buffer, protein-buffered NaCl solution; ready to use, blue color
Wash	100 ml	Wash buffer, phosphate buffered NaCl solution (concentrated 10-fold); contains 0.1% thimerosal
Control | +	2 ml	Positive control (inactivated *Campylobacter* antigen; ready for use
Control | -	2 ml	Negative control (sample dilution buffer); ready for use
Conjugate | 1	13 ml	Biotin-conjugated antibodies to *Campylobacter jejuni* and *Campylobacter coli* in stabilized protein solution; ready for use; blue color
Conjugate | 2	13 ml	Streptavidin poly-peroxidase conjugate in stabilized protein solution; ready for use; orange colored
Substrate	13 ml	Hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop reagent; 1 N sulphuric 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 torn off. Any microwell 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. Additional necessary reagents – and necessary equipment

6.1. Reagents

- Distilled or deionized water

6.2. Equipment

- Test tubes
- Disposable pipettes (Article no.: Z0001)
- Vortex mixer (optional, see 9.3.)
- Micropipette for 50–100 µl and 1 ml volumes
- Measuring cylinder (1000 ml)
- Timer
- 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 0.5% hypochlorite solution

7. Precaution 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. Always adhere strictly to the user instructions for this test.

Specimens or reagents must not be pipetted by mouth, and contact with injured skin or mucous membranes must be prevented. Wear personal safety gear (suitable gloves, laboratory coat, safety glasses) when handling the specimens, and wash hands after finishing the test. Do not smoke, eat, or drink in areas where samples are being processed.

For more details, refer to Material Safety Data Sheets (MSDS) at www.r-biopharm.com.

The kit includes a positive control that contains the inactivated *Campylobacter* antigen. That, just like the patient samples, must be treated as potentially infectious material and handled in accordance with national safety regulations.

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

Ensure the proper and responsible disposal of all reagents and materials after their use. For disposal, please adhere to national regulations.

8. Specimen collection and storage

Until it is used, store the test material at 2 – 8 °C. If the material cannot be used for a test

within three days, we recommend storage at -20 °C or colder. Avoid freezing and thawing the specimen repeatedly. After diluting a stool sample in sample dilution buffer 1:11, it can be stored at 4 °C for use within seven days.
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® Campylobacter Test.
Stool samples packed in the commonly marketed transport media (Cary Blair, Amies) can be used in the RIDASCREEN® Campylobacter ELISA. However, the here required pre-dilution of the sample must be taken into account. As far as possible, the end dilution of the stool sample in Diluent 1 should be precisely 1:11.
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 procedures

9.1. General information

All reagents and the microwell 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 used again. 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

Fill a labelled test tube with 1 ml RIDASCREEN® sample dilution buffer Diluent 1. Use a disposable pipette (article no. Z0001) to aspirate a sample of thin stool (approx. 100 µl) to just above the second bulge and add this to buffer in the test tube to make a suspension. To make a suspension with a solid stool sample, add an equivalent amount (approx. 50100 mg) with a spatula or disposable inoculation loop.
Homogenize the stool suspension by aspiration and ejection from a disposable pipette, or, alternatively, by mixing in a vortex mixer.

Let the suspension stand a short period of time for the coarse stool particles to settle; this clarified supernatant of the stool suspension can be 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.

Note:

Stool samples diluted in Diluent | 1 can be used in any other RIDASCREEN® ELISA, provided that it also uses Diluent | 1.

9.4. First incubation

After filling a sufficient number of wells in the strip holder, add 100 µl of the positive Control | +, the negative Control | - or the stool sample suspension (or, if available, the supernatant of the colony suspension) to the wells. Subsequently add 100 µl of the biotin-conjugated antibody Conjugate | 1 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 proceed strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container for disposal in accordance with local regulations. After this, knock 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 knocking them out after each wash on a part of the absorbent paper which is still dry and unused.

If you use a microplate washer or fully automated ELISA, make sure that the machine is correctly adjusted; request 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 make sure that all of the liquid is aspirated during each wash step.

9.6. Second incubation

Use a pipette to fill 100 µl streptavidin poly-HRP conjugate Conjugate | 2 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, measure the extinction at 450 nm (optional: 450/620 nm). Adjust the zero point in the air that is without the microwell plate.

Note:

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

9.9 Abridged test protocol

The incubation times described under Items 9.4 and 9.6 can be significantly shortened, if the plate is incubated at 37 °C and a vibration frequency of 20–25 Hz (DSX®, Fa. Dynex). The incubation times change to the following:

Incubation 1: 30 min
Incubation 2: 15 min
Incubation 3: 15 min

Separate microwell plate shakers are also suitable, such as the Thermomixer by Eppendorf (frequency setting: 850 rpm) or also DTS2 by LTF Labortechnik (frequency setting 800 rpm).

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 reagents are stable and that the test is conducted correctly. The test has been carried out correctly if the extinction rate (O.D.) 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

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = extinction for the negative control + 0.15

11.2. Test results

Assessment of the specimen is positive if the extinction rate is more than 10 % higher than the calculated cut-off value.
Assessment of the specimen is marginal 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 with extinctions more than 10 % below the calculated cut-off must be considered negative.

12. Limitations of the method

The RIDASCREEN® Campylobacter Test identifies the specific antigens of *Campylobacter jejuni* and *Campylobacter coli* in stool samples. 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 result does not rule out the presence of other infectious pathogens.
A negative result does not rule out the possibility of *C. jejuni* or *C. coli* infection. 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 infection with *C. jejuni* or *C. coli*, the examination should be repeated with another stool sample.
A marginal result may be due to non-homogeneous distribution of the antigens 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.

13. Performance characteristics

13.1. Test quality

The performance of the RIDASCREEN® Campylobacter ELISA was evaluated in a comparison study with the gold standard, i.e. cultivation of the pathogen on CCD agar under microaerophilic conditions. RIDASCREEN® Campylobacter ELISA was used to test a total of 574 stool samples from the routine diagnostic practice of a participating laboratory. The results of that study are summarized in Table 1.

Table 1: Comparison of RIDASCREEN® Campylobacter against cultivation on CCDA plates

		Culture (CCDA)	
		Positive	Negative
RIDASCREEN® Campylobacter	Positive	61	4
	Negative	2	507

Sensitivity:	96.8 %	Positive predictive value (PPV):	93.8 %
Specificity:	99.2 %	Negative predictive value (NPV):	99.6 %

13.2. Analytical sensitivity

The analytical limits of detection of *C. jejuni* and *C. coli* were determined separately. It describes the lowest pathogen concentration which could still be positively identified in the RIDASCREEN® Campylobacter ELISA (>10% above calculated cut-off). Calculated on the basis of 90 trials (CI ≥ 95%) the cut-off for *Campylobacter jejuni* is 1.9×10^4 CFU/ml, and for *Campylobacter coli* it is 1.1×10^6 CFU/ml.

13.3. Cross reactivity

A variety of pathogenic microorganisms from the intestinal tract were examined with the RIDASCREEN® Campylobacter ELISA and showed no cross reactivity. These tests were conducted with bacterial suspensions (10^6 to 10^9 CFU/ml), with parasite cultures (10^7 to 10^9 organisms/ml) and with cell culture supernatants from virus infected cells. The results of that study are listed in Table 2. With the exception of the two Campylobacter species *Campylobacter jejuni* and *Campylobacter coli*, none of the organisms in the test reacted in the RIDASCREEN® Campylobacter ELISA.

Table 2: Cross reactivity with pathogenic microorganisms from the intestinal tract

Organism	**Origin/source**	**[OD 450]**
Adenovirus	Infectious culture supernatant	0.086
Aeromonas hydrophila	Culture	0.081
Astrovirus	Infectious culture supernatant	0.068
Bacillus cereus	Culture	0.070
Bacteroides fragilis	Culture	0.067
Campylobacter coli	Culture	2.007
Campylobacter fetus	Culture	0.066
Campylobacter jejuni	Culture	3.622
Campylobacter lari	Culture	0.074
Campylobacter upsaliensis	Culture	0.073
Candida albicans	Culture	0.062
Citrobacter freundii	Culture	0.063
Clostridium difficile	Culture	0.055
Clostridium perfringens	Culture	0.055
Clostridium sordellii	Culture	0.060
Cryptosporidium muris	Culture	0.062
Cryptosporidium parvum	Culture	0.060
E. coli (O26:H-)	Culture	0.058
E. coli (O6)	Culture	0.054

E. coli (O157:H7)	Culture	0.054
Enterobacter cloacae	Culture	0.053
Enterococcus faecalis	Culture	0.059
Giardia lamblia sample	stool specimen	0.078
Klebsiella oxytoca	Culture	0.057
Proteus vulgaris	Culture	0.054
Pseudomonas aeruginosa	Culture	0.056
Rotavirus	Infectious culture supernatant	0.066
Salmonella enteritidis	Culture	0.046
Salmonella typhimurium	Culture	0.050
Serratia liquefaciens	Culture	0.047
Shigella flexneri	Culture	0.048
Staphylococcus aureus	Culture	0.051
Staphylococcus epidermidis	Culture	0.052
Vibrio parahaemolyticus	Culture	0.053
Yersinia enterocolitica	Culture	0.046

13.4. Precision

To determine the precision of the assay, six samples were used, each with a defined extinction level (OD) which covered the whole measurement range of the test. Table 3 presents the OD spectra of the samples.

Table 3: Extinction spectra of the samples

Samples	OD spectra
Sample 1	1.387 - 2.575
Sample 2	0.946 - 1.758
Sample 3	0.693 - 1.287
Sample 4	0.470 - 0.874
Sample 5	0.370 - 0.686
Sample 6	0.281 - 0.523

The reproducibility of the RIDASCREEN® Campylobacter ELISA was tested with the six listed references representing the complete measurement range from weak to high positive. To determine the intra-assay reproducibility, 39 replicates of these references were assayed. The mean values and the variation coefficients (VC) were determined for three lots of the kits. For the inter-assay reproducibility, samples from ten sequential working days were assayed in

duplicates, with two runs per day. The measurements were determined in three lots by three technicians. The inter-lot reproducibility was determined for all three kit lots. The results of that study are summarized in Table 4.

Table 4: Mean values and variation coefficients of the six references

Samples 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
Sample 1	1.625 / 7.18%	2.487 / 4.33%	2.269 / 5.94%	1.993/ 12.17%	2.188 / 13.46%	2.273 / 12.80%	2.151/ 14.24%
Sample 2	1.085 / 7,79%	1.588 / 5.78%	1.444 / 5.33%	1.389 / 15.88%	1.551/ 13.84%	1.619 / 13.93%	1.519 / 16.17%
Sample 3	0.779/ 6.71%	1.149 / 6.90%	1.171 / 5.51%	0.977 / 11.02%	1.095 / 13.28%	1.166 / 16.34%	1.079 / 16.32%
Sample 4	0.539/ 6.18%	0.710 / 6.27%	0.707 / 6.91%	0.638 / 12.46%	0.689/ 15.74%	0.804 / 17.27%	0.710 / 19.50%
Sample 5	0.397 / 9.27%	0.543/ 4.95%	0.710 / 5.45%	0.483/ 15.50%	0.541 / 17.52%	0.624/ 17.96%	0.549 / 21.24%
Sample 6	0.272 / 11.76%	0.391/ 5.89%	0.478 / 10.03%	0.381 / 19.99%	0.417 / 16.87%	0.469 / 21.18%	0.422 / 21.87%

14. Interfering substances

The following list of substances showed no significant effects on the test results when they were blended into Campylobacter positive and Campylobacter negative stool samples in the described concentrations**:**

barium sulfate (5% w/w), loperamide (antidiarrheal drug; 5% w/w), Pepto-Bismol (antidiarrheal drug, 5% v/w), mucins (5% w/w), cyclamate (artificial sweetener, 5% v/w), human blood (5% v/w), stearic acid and palmitinic acid (mixture 1:1, 20% w/w), metronidazole (0.5) (antibiotic 5% v/w), diclofenac (0.00263% v/w).

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. Skirrow MB, Blaser MJ (1995): Campylobacter jejuni. In: Blaser MJ,Smith PD, Ravdin JI, Greenberg HB, Guerrant RL (eds) Infections of the Gastrointestinal Tract. Raven Press, New York, 825-248.
2. Kist M, Bereswill S (2001) Campylobacter jejuni. In: Mühldorfer I, Schäfer KP (eds) Emerging bacterial pathogens. Contrib Microbiol Vol.8. Karger, Basel, 150-165.
3. Skirrow MB (1977) Campylobacter enteritis: a new disease. Br Med J 2: 9-11.
4. Rees JH, Soudain SE, Gregson NA, Highes RA (1995) Campylobacter jejuni infection and Guillain-Barré syndrome. N Engl J Med 333: 1374-1379.
5. Beumer RR, Cruysen JJ, Birtantie IR (1988) The occurrence of Campylobacter jejuni in raw cow milk. J Appl Bacteriol 65: 93-96.
6. Jones K (2001) Campylobacters in water, sewage and the environment. J Appl Microbiol 90: 68 S-79 S
7. Skirrow MB, Turnbull GL, Walker RE, Young SEJ (1980) Campylobacter jejuni enteritis transmitted from cat to man. Lancet 1: 1188.
8. Steinbrückner B, Härter G, Pelz K, Kist M (1999) Routine identification of Campylobacter jejuni and Campylobacter coli from human stool samples. FEMS Microbiol Lett 179: 227 –232.
9. Kist M (2002) Lebensmittelbedingte Infektionen durch Campylobacter. Bundesgesund-heitsbl-Gesundheitsforsch- Gesundheitsschutz 45: 497-506.