Clostridium perfringens Enterotoxin ELISA KIT

Cat. No.:DEIA05710
Pkg.Size:96T

Intended use

Clostridium perfringens Enterotoxin is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of Clostridium perfringens enterotoxin in stool samples.

General Description

Like all other clostridia, Clostridium perfringens (CP) is a ubiquitous, spore-forming, gram-positive, anaerobic bacterium that is usually present in the colonic flora of humans. The absence of peritrichous flagella distinguishes C. perfringens from other Clostridium species. Clostridium perfringens species are divided into 5 different types (A-E). In humans, necrotizing enteritis is mainly caused by type A and, under certain circumstances (diet or trypsin deficiency), by type C, but all types of C. perfringens can cause the disease in animals.

Strains are classified as types A-E depending on their ability to produce the four major C. perfringens toxins (alpha, beta, epsilon and iota), all of which have cytotoxic and necrotic effects. Besides the four main toxins, C. perfringens strains may also produce a number of other toxins, including enterotoxin, a 35 kDa polypeptide, which is of pathological significance in humans.

Food poisoning induced by C. perfringens enterotoxin (CPE) is the longest known form of C. perfringens infection. The consumption of food that was improperly cooked or stored, especially if eaten some time after preparation, may result in the ingestion of large quantities of C. perfringens. When this occurs, the pathogens produce large amounts of toxins, resulting in intestinal disorders such as diarrhea and abdominal cramps. The symptoms start about 8 to 24 hours after consumption of the contaminated food and subside within another 24 hours. The diarrhea is occasionally accompanied by vomiting, fever and headache. Besides causing CPE food poisoning, C. perfringens is increasingly being recognized as a cause of antibiotic-associated diarrhea (AAD). It is now implicated in approximately 10% of cases of antibiotic-associated diarrhea and in 5-20% of cases of sporadic diarrhea (SPOR) caused by non-food-borne organisms. These forms of diarrhea may result in complications as severe as pseudomembranous colitis. They have a much more severe and protracted clinical course (10 to 30 days) and are often accompanied by blood and mucus in the stools. Histological sections of the lower large intestine and rectum show areas of edema produced by lesions caused by C. perfringens enterotoxin.

Recent studies have shown that while the gene encoding C. perfringens enterotoxin may either chromosomal or episomal, the amino acid sequence contained in the two variants is the same. Furthermore, it was found that the CPE isolates involved in food poisoning always form chromosomally encoded CPE, and that those involved in antibiotic-associated and sporadic diarrhea always form plasmid-encoded CPE.

The reason why C. perfringens enterotoxin has a much more severe impact on AAD and SPOR patients is probably that these carriers tend to be patients whose health is already impaired by another illness, old age, or a weakened immune system. Older people, particularly those living in nursing homes, and hospital patients tend to have two detrimental factors: 1) an increased susceptibility to infection and 2) a large number of persistent carriers of Clostridium perfringens.

Alongside Clostridium difficile Toxin A/B, Clostridium perfringens Enterotoxin is an important tool for the diagnosis of antibiotic-associated and sporadic diarrhea. The CPE test can help in establishing a quick and reliable diagnosis and, thus, in treatment decision-making.

Principle Of The Test
Clostridium perfringens Enterotoxin is an enzyme-linked immunosorbent assay (ELISA) that uses monoclonal antibodies based on the sandwich principle. Monoclonal antibodies against epitopes of C. perfringens enterotoxin are bound to the surface of the wells of the microplate. The diluted test sample, the positive control and the negative control are dispensed into the respective wells of the microwell plate together with biotinylated anti-enterotoxin antibodies (Conjugate 1) and incubated at room temperature (20-25 °C). After a washing step, a streptavidin-peroxidase conjugate (Conjugate 2) is added and the plate is incubated a second time at room temperature (20 to 25 °C). The presence of enterotoxin in the sample results in the formation of a sandwich complex of immobilized antibodies, enterotoxin and conjugated antibodies. Excess unbound streptavidin peroxidase is then removed in an additional washing step. If the sample is positive, the addition of Substrate Solution will result in enzyme binding, which is reflected as a change of color of the solution in the microwells from colorless to blue. After the addition of Stop Solution, the color changes from blue to yellow. The subsequently measured extinction is proportional to the concentration of CPE in the sample.

### Reagents And Materials Provided

Each kit contains sufficient reagents for 96 tests.

1. **Plate** 96 tests-Microwell Plate: Eight 12-microwell strips (separable) in strip holder with wells coated with specific antibodies to Clostridium perfringens enterotoxin.
2. **Diluent | 1 100 ml-Sample Diluent:** Protein-buffered NaCl solution, ready for use, blue in color.
3. **Wash 100 ml-Wash Buffer:** Phosphate-buffered NaCl solution (10X concentrate); contains 0.1 % thimerosal.
4. **Control | + 1.8 ml-Inactivated enterotoxin, ready for use.
5. **Conjugate | 1 10 ml-Conjugate 1:** Biotin-conjugated antibodies to enterotoxin in stabilized protein solution, ready for use; blue in color.
6. **Conjugate | 2 10 ml-Conjugate-2:** Streptavidin-peroxidase conjugate in stabilized protein solution, ready for use; orange in color.
7. **Substrate 10 ml- Substrate Solution:** Hydrogen peroxide/TMB, ready for use.
8. **Stop 6 ml- Stop Solution:** 1 N sulfuric acid, ready for use.

### Materials Required But Not Supplied

1. **Reagents**  
   - Distilled or deionized water
2. **Equipment**  
   - Test tubes  
   - Disposable pipettes  
   - Vortex mixer (optional)  
   - Micropipette for 50 to 100 µl and 1 ml volumes  
   - Graduated cylinder (1000 ml)  
   - Stopwatch  
   - Washing unit for microwell plates or multichannel pipettes (300 µl)  
   - Photometer for microwell plates (450 nm, reference filter 620-650 nm)  
   - Filter paper (laboratory tissues)  
   - Waste receptacle

### Storage

All reagents must be stored at 2 to 8 °C and can be used until the expiration date printed on the product label. After
reconstitution, diluted wash buffer can be stored for 4 weeks at 2 to 8 °C. Avoid microbial contamination! The quality of the product cannot be guaranteed after the expiration date.

Use scissors to carefully open the resealable foil pouch without cutting off the clip seal. Immediately return unused microwell strips to the foil pouch and store in the sealed pouch at 2 to 8 °C.

Protect the colorless Substrate Solution from direct light exposure in order to prevent decomposition and auto-oxidation, as reflected by blue discoloration of the solution. Do not use Substrate Solution if discolored (blue).

**Specimen Collection And Handling**

All test specimens must be stored at 2 to 8 °C until processed for testing. If the samples cannot be processed within 3 days, they should be stored at –20 °C or colder. Repeated freezing and thawing of samples should be avoided.

Stool samples or rectal swabs should not be collected in transport containers using transport media containing preservatives, animal sera, metal ions, oxidizing agents or detergents as these substances may interfere with the Clostridium perfringens Enterotoxin assay.

If rectal swabs are to be used, ensure that sufficient fecal matter (ca. 100 mg) is available for performance of the assay. When conducting environmental studies, stool samples should also be taken from clinically normal persons who have come in contact with the pathogen in order to identify asymptomatic carriers.

**Assay Steps**

1. General information

Allow kit reagents and microwell plate **Plate** to reach room temperature before use (20 to 25 °C). Do not remove the microwell strips from the foil pouch until they have reached room temperature. The reagents should be thoroughly mixed immediately prior to use. After use, return all unused microwell strips and reagents to the refrigerator and store at 2 to 8 °C (in sealed pouch).

Each microwell strip is for single use only and cannot be reused. Do not use regents and microwell strips if the packaging is damaged or the vials are not sealed properly.

To prevent cross-contamination, do not allow test samples to come in direct contact with the kit components. Do not perform the test in direct sunlight. The microwell plate should be covered or taped during testing to avoid evaporation loss.

2. Preparation of Wash Buffer

Mix 1 part concentrated Wash Buffer **Wash** with 9 parts distilled water. Any crystals present in the concentrate must be dissolved by warming the vial in a warm water bath (37 °C) before reconstitution.

3. Sample preparation

Dispense 1 ml of Sample Diluent 1 **Diluent | 1** into a labeled test tube. Liquid stool specimens are drawn into a disposable pipette to just above the second bulge (ca. 100 µl) and added to the sample diluent in the test tube to yield a suspension. When processing solid fecal specimens, an equivalent amount of fecal material (100 mg) is collected with a spatula or disposable inoculating loop and added to the sample diluent in the test tube to yield a suspension.

Homogenization of stool suspensions is accomplished either by drawing the stool suspension into and out of the disposable pipette or by mixing it on a vortex mixer. After homogenization, allow the stool suspension to stand briefly to ensure the sedimentation of coarse fecal particles. The thus clarified supernatant of the stool suspension can be directly used for testing. The supernatant must be particle-free if the assay is to be run on an automated ELISA system. In this case, it is recommended to centrifuge the supernatant at 2500 G for 5 minutes.

4. First incubation

After inserting the desired number of coated wells in the microwell holder frame, dispense 2 drops (100 µl) of Positive Control **Control | +**. Sample Diluent Diluent | 1 (= Negative Control), and diluted stool sample (or supernatant of the colony suspension) into the respective wells. Then add 2 drops (100 µl) of biotin-conjugated antibody **Conjugate | 1**, mix (by gently tapping the side of the plate), and incubate at room temperature (20 to 25 °C) for 60 minutes.
5. Washing
Thorough washing of the plate is crucial for obtaining correct test results, so strict adherence to instructions is essential. First, the incubated liquid in the wells should be emptied into a waste receptacle and disposed of in accordance with national safety regulations. The next step is to tap the plate on absorbent paper to remove the remaining liquid and wash the plate 5 times using 300 µl of Wash Buffer per washing step. After each washing step, tap the plate on a dry, unused part of the absorbent paper to ensure the complete removal of liquids.
When using an automated washer or a fully automated ELISA system, ensure that the machine is correctly set for the specific type of plate being used. The systems supplied by R-Biopharm are preprogrammed with validated settings and work protocols. To prevent blockage of the washing needles, all stool suspensions processed on the system should be completely particle-free (see Section 3). Ensure that the solutions are completely removed during each washing step.

6. Second incubation
Dispense 2 drops (100 µl) of the streptavidin-peroxidase conjugate Conjugate | 2 into each well and incubate at room temperature (20 to 25 °C) for 30 minutes.

7. Washing
Wash as directed in Section 5.

8. Third incubation
Dispense 2 drops (100 µl) of Substrate Solution Substrate into each well and allow the plate to incubate in the dark at room temperature (20 to 25 °C) for 15 minutes. After incubation, add 1 drop (50 µl) of Stop Solution Stop to each well to terminate the reaction. After mixing carefully (by gently tapping the side of the plate), measure the extinction/optical density (OD) in the wells at 450 nm (optional wavelength: 450/620 nm). The plate reader should be blanked on air, i.e., without a microwell plate.

**NOTE:** Strong positive reactions can result in the formation of black precipitates from the substrate.

### Quality Control
For quality control purposes, both the positive and negative control must be assayed with each batch of patient specimens to ensure reagent stability and proper test performance. The assay has been carried out correctly if the optical density (OD) of the negative control is less than 0.2 at 450 nm (< 0.160 at 450/620 nm), and that of the positive control is greater than 0.8 at 450 nm or 450/620 nm. Negative control OD values greater than 0.2 (> 0.160) may be a sign of insufficient washing. Deviation of the controls from the target optical density range and cloudiness or blue discoloration of the colorless Substrate Solution before use may indicate reagent deterioration. If the measured OD values lie outside the target ranges, the following checks should be performed before repeating the assay:
- Check the expiration date of the reagents.
- Conduct a functional check of the equipment (e.g., calibration).
- Check to determine whether the test has been performed correctly.
- Visually inspection kit components for contamination, leaks and discoloration; do not use Substrate Solution if discolored (blue).
If quality standards still are not met after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

### Calculation
**Calculation of the cut-off value**
The cut-off value is calculated by adding 0.15 extinction units to the measured optical density (OD) of the negative control.
Cut-off = Optical Density of Negative Control + 0.15

### Interpretation of Results
Samples with OD values greater than 10 % higher than the calculated cut-off are classified as **positive**. Samples with OD values ranging from equal to 10 % greater than to equal to 10 % less than the cut-off are classified as
borderline and must be re-tested. If the OD value of a repeat test on a fresh stool sample should also lie in this gray area, the sample should be interpreted as negative. Samples with OD values greater than 10% below the calculated cut-off value are classified as negative.

### Specificity

Various pathogenic bacteria of the intestinal tract were tested with the Clostridium perfringens Enterotoxin ELISA and exhibited no cross-reactivity. The tests were performed using bacteria suspensions (10^6 to 10^9 CFU/ml), parasite cultures (10^7 to 10^9 organisms/ml), and culture supernatants of virus-infected cells. The results are summarized below in Table.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Result [OD 450nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>0.050</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>0.046</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0.054</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>0.053</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>0.060</td>
</tr>
<tr>
<td>Campylobacter fetus</td>
<td>0.055</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>0.053</td>
</tr>
<tr>
<td>Campylobacter lari</td>
<td>0.060</td>
</tr>
<tr>
<td>Campylobacter upsaliensis</td>
<td>0.051</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.052</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>0.052</td>
</tr>
<tr>
<td>Clostridium bifermentans</td>
<td>0.044</td>
</tr>
</tbody>
</table>
Reproducibility of the Clostridium perfringens Enterotoxin ELISA was tested using six reference samples that cover the entire spectrum from negative to highly positive (see below). Intra-assay reproducibility was determined using 40 replicates of these reference samples. Mean and coefficient of variation (CV) values were determined from three kit lots. Inter-assay reproducibility was determined using the reference samples, which were assayed in duplicate in two separate runs per day on 10 consecutive work days. The measurements were performed by two laboratory technicians using three kits lots. Inter-lot reproducibility was averaged over all three kit lots.
Interferences

The following substances were tested with the Clostridium perfringens Enterotoxin ELISA and exhibited no significant effect on the test results when mixed with CPE-positive and CPE-negative stool samples at the specified concentrations: Barium sulfate (5 % w/w), loperamide (antidiarrheal, 5 % w/w), Pepto-Bismol (antidiarrheal, 5 % v/w), mucin (5 % w/w), cyclamate (artificial sweetener, 5 % v/w), human blood (5% v/w), stearic acid / palmitic acid (1:1 ratio, 40% w/w), metronidazole (0.5) (antibiotic, 5 % v/w), and diclofenac (0.00263% v/w).

Precautions

1. This assay should only be performed by trained laboratory personnel. The rules of good medical laboratory practice should be followed. Strict adherence to the test instructions is advised.
2. The positive control provided with the kit contains inactivated C. perfringens enterotoxin. These kit components and patient samples must be treated as potentially infectious materials and handled in accordance with national safety regulations.
3. Do not pipette samples or reagents by mouth and avoid contact of reagents with injured skin or mucous membranes. Wear disposable gloves when handling samples and wash hands after completion of testing. Do not smoke, eat or drink in areas where samples or kit reagents are handled.
4. Wash Buffer contains 0.1 % thimerosal as a preservative. Avoid contact with skin and mucous membranes.
5. Hydrogen peroxide (TMB) is caustic and can cause burns. Handle with care!
6. Stop Solution contains 1 N sulfuric acid. Avoid contact with skin and clothing! Skin exposed to the acid should be rinsed with water.
7. All reagents and materials coming in contact with potentially infectious samples must be treated with appropriate disinfectants or autoclaved at 121 °C for at least one hour. WARNING: To prevent the formation of poisonous gases, any liquid waste containing Stop Solution must be neutralized prior to disposal.

Limitations

Clostridium perfringens Enterotoxin is an ELISA for the qualitative analysis of Clostridium perfringens enterotoxin in stool samples. No correlation between the measured OD values and the occurrence or severity of clinical symptoms can be deduced from this test. The test results must always be interpreted in conjunction with the clinical signs and symptoms. A positive result does not rule out the possibility that other infectious agents may also be present. A negative result does not rule out the possibility of C. perfringens infection. The test may be negative due to the proteolytic degradation of the enterotoxin or to improper storage of the sample. If there is justified suspicion of C. perfringens infection
based on the case history, another stool sample should be collected and tested.

A **borderline** result may occur due to the uneven distribution of toxins in the stool sample. If the test results are borderline, the same stool specimen should be resuspended and re-tested or a second stool specimen should be collected and tested.

**REFERENCES**