Clostridium perfringens beta toxin

ELISA kit for antigenic detection of
Clostridium perfringens beta toxin
Sandwich test for culture supernatants and biological fluids
Diagnostic test for all species
Double wells

I - INTRODUCTION

Enterotoxaemia is a fatal enteric disease that affects all species of domestic animals and is attributable to a toxigenic type of *Clostridium perfringens*. The latter is an anaerobic, strongly gram-positive bacterium that has the ability to form heat-resistant endospores. This bacterium is grouped into five types (types A, B, C, D and E) according to the four major lethal toxins, Alpha, Beta, Epsilon, and Iota (α, β, ε, ι) produced.
Clostridium perfringens has been shown to be a cause of human diseases such as gas gangrene (clostridial myonecrosis), food poisoning, necrotising enterocolitis of infants, and enteritis necroticans (pigbel). It is also the causative agent of lamb dysentery, ovine enterotoxaemia (struck) and pulpy kidney disease of sheep, and other enterotoxaemic diseases of lambs and calves. Large amounts of toxin in addition to large numbers of *Clostridium perfringens* cells can usually be detected in the intestinal fluid of the diseased or dead animals.
As *Clostridium perfringens* is a natural commensal of human and animal intestines, identifying of the bacterium is not enough. Toxinotyping and quantifying of the isolated strains are essential.
The kit works with culture supernatants as well as biological probes such as liquid intestinal contents and pericardial- or peritoneal fluid.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by specific monoclonal antibodies for the Beta toxin. These antibodies allow a specific capture of the corresponding antigen which is present in the samples. Rows A, C, E, G have been sensitized with these antibodies and rows B, D, F, H are containing non specific antibodies. These control rows allow the differenciation between specific immunological reaction and non specific bindings.
Biological samples (for example: contents of the small intestine, peritoneal fluid....) are diluted in dilution buffer and incubated on the microplate for 1 hour at 21°C +/- 3°C. Culture supernatants are used without any dilution.
After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate - a peroxidase labelled anti-Beta-toxin specific monoclonal antibody. After this second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.
If Beta-toxin is present in the tested samples, the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity

of the resulting blue colour is proportionate to the titre of Beta-toxin in the sample. Enzymatic reaction can be stopped by acidification and resulting optical density at 450 nm can be recorded using a photometer. The signals recorded for the negative control microwells are substracted from the corresponding positive microwells.
There is a positive antigen supplied with the kit.

TOXINOTYPES

Toxinotypes	Alpha	Beta	Epsilon	Iota
A	++	-	-	-
B	+	++	+	-
C	+	++	-	-
D	+	-	++	-
E	+	-	-	++

III - COMPOSITION OF THE KIT

- **Microplates:** 96-well microtitration plate (12 Strips x 8 wells). Rows A, C, E, G are sensitised by anti-Beta-toxin specific antibodies, while rows B, D, F, H are sensitized by the non specific antibodies.
- **Washing solution:** bottle concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.
- **Dilution buffer:** One bottle of 5x colored, concentrated buffer for diluting samples. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: vial of anti-Beta-toxin-peroxidase colored conjugate. **This solution is ready to use.**
- **Control antigen**: This reagent is ready to use.
  Single component TMB: bottle of the chromogen tetramethylbenzidine (TMB). Store at + 2°C and + 8°C protected from light. **This solution is ready to use.**
- **Stopping solution**: bottle of the 1 *M* phosphoric acid stop solution.

	BIO K 267/1	BIO K 267/2
Microplates	1	2
Washing dilution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 50 ml (5 X)	1 X 50 ml (5 X)
Conjugate	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Control antigen	1 X 2 ml (1 X)	1 X 4 ml (1 X)
Single component TMB	1 X 12 ml (1 X)	1 X 25 ml (1 X)
Stopping solution	1 X 6 ml (1 X)	1 X 15 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

1- Bring all the reagents at 21°C +/- 3°C before use.

2- Dilute the concentrated washing solution 20 fold in distilled water. Be sure that all crystals have disappeared before dilution.
Dilute the concentrated Dilution buffer 5 fold in distilled water.
Keep these solutions at + 2°C and + 8°C when not used.

3- Dilute the samples volume per volume into dilution buffer prepared as instructed in §2. This is a qualitative dilution only, which must allow the pipetting of biological suspensions. Discard eventual gruds by natural decantation for about 10 minutes. Do not centrifuge the suspensions. Culture supernatants are used undiluted. The best results have been obtained by using liquid TGY under anaerobic conditions (in a tube without shaking) at 37°C. The samples may be cultured for 8 hours or overnight.

Composition of TGY*:*

- Trypticase (casein tryptic peptone):	30 g
- Yeast extract:	20 g
- Glucose:	1 g
- L-cysteine :	1 g

Dissolve Trypticase and Yeast extract in 950 ml of water and autoclave. Dissolve glucose and L-cysteine in 50 ml of water and sterilise by filtration. Mix the two solutions when the first one is at 21°C +/- 3°C.

4- Add 100-µl aliquots of the diluted samples or the non diluted supernatants to the wells as follows: Sample 1 in wells A1 and B1, sample 2 in wells C1 and D1, etc. Proceed in the same manner for the positive reference (ex.: G1 and H1)

5- Cover with a lid and incubate the plate at 21°± 3°C for one hour.

6- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.

7- Distribute the conjugate solution at the rate of 100 µl per well. Cover with a lid and incubate the plate at 21°± 3°C for one hour.

8- Wash the plate as described in Step 6.

9- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.

Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover. This time interval is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.

10- Add 50 µl of stop solution to each well. The blue colour will change into a yellow colour.

11- Read the optical densities by means of a microplate spectrophotometer with a 450 nm filter. The results must be read as quickly as possible after the stop solution has been applied, for in the case of a strong signal the chromogen can crystallise and lead to incorrect measurements

VII – INTERPRETING THE RESULTS

Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control.
Proceed in the same way for the positive control antigen.
The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the QC data sheet.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage. Using the first table in the quality control procedure, determine each sample's status (positive, negative).

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

VIII – ORDERING INFORMATION

Monoscreen AgELISA *Clostridium perfringens* beta toxin	1 X 48 tests	BIO K 267/1
	2 X 48 tests	BIO K 267/2