MONOSCREEN Ab ELISA

Clostridium perfringens epsilon toxin

ELISA kit for serodiagnosis of *Clostridium perfringens* Epsilon toxin
Blocking test for blood sera and plasma
Diagnostic test for all species
Monowell

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. *C.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 *C.perfringens* cells can usually be detected in the intestinal fluid of the diseased or dead animals. As *C. 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 BIO K 222 test is designed to monitor the animal's serological response after immunisation by a vaccine or natural contact with *Clostridium perfringens*. As it is a blocking test, it can be used in all animal species.

II - PRINCIPLE OF THE TEST

The 96-well microplate has been sensitised by a polyclonal antibody specific for *C. perfringens* epsilon toxin.
C. perfringens epsilon toxin was then added to these microplates. The kit's user deposits the previously diluted test sera and plasma in the microplate's wells. After 2 hours' incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against *C. perfringens* epsilon toxin coupled to peroxidase. After incubating and washing the preparation, the operator adds the chromogen tetramethylbenzidine (TMB). This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the colour is inversely proportionate to the sample's serum titre. Positive and negative sera are provided with the kit to be able to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plates. The entire surface of each microplate has been sensitised with *C. perfringens* epsilon toxin.
- **Washing solution**: One bottle of 20x 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. Store the diluted solution between +2°C and +8°C.
- **Dilution buffer**: One bottle of colored buffer for diluting samples and conjugate. The dilution buffer is ready to use. Store the solution between + 2°C and + 8°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: 1 vial of anti - *C.perfringens* epsilon toxin peroxidase conjugate (horseradish peroxidase-labelled anti- *C. perfringens* epsilon toxin monoclonal antibody). The reagent must be diluted 1:20 with dilution buffer.
- **Positive reference**: One bottle of positive serum. Store this reagent between +2°C and +8°C.
- **Negative reference**: One bottle of negative serum. Store this reagent between +2°C and +8°C.
- **Single component TMB**: One bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
- **Stopping solution**: One bottle of the 1 M phosphoric acid stop solution.

	BIO K 222/1	BIO K 222/2
Microplate	1	2
Washing solution	1 X 100 ml (20 X)	1 X 100 ml (20 X)
Colored Dilution buffer	1 X 60 ml (1X)	1 X 60 ml (1X)
Conjugate	1 X 0.625 ml (20X)	1 X 1.250 ml (20X)
Positive serum	1 X 0.5 ml (1 X)	1 X 0.5 ml (1 X)
Negative serum	1 X 0.5 ml (1 X)	1 X 0.5 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 may be stored at room temperature. Once diluted, this solution remains 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 components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.

2- PREPARATION AND DILUTION OF SAMPLES

The blood serum or plasma samples must be diluted twofold. Avoid using haemolysed samples or samples that contain coagulum.

Deposit 50 µl of buffer dilution directly into the wells of the kit's microplate. Add 50 µl aliquots of each sample to each well. Do the same for the reference sera (positive and negative controls). Cover with a lid and incubate the plate at 37°C for 2 hours.

3- 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.

4- Dilute the conjugate 1:20 in the dilution buffer (for example, for one plate dilute 600 µl of the conjugate stock solution in 11.400 ml of diluent).
Add to each well used 100 µl of the conjugate. Cover with a lid and incubate the plate at 37°C for 1/2 hour.

5- Rinse the plate with the washing solution as instructed in step 3.

6- 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.

7- Incubate for 10 minutes at 21°C +/- 3°C protected from the light and uncovered. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.

8- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.

9- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallise in wells with strong signals and distort the results accordingly.

VII – INTERPRETING THE RESULTS

Measure the optical densities of the positive and negative sera (OD pos and OD neg) and those of all the samples (OD samples).
Calculate the percent inhibition (% inhib) for each tested sample and the positive serum by means of the following formulas:

% inh sample = [(OD neg – OD sample)/OD neg]*100
% inh positive = [(OD neg – OD pos)/OD neg]*100

VIII – VALIDATING THE TEST

The test may be validated only if the following two conditions are met:

- OD neg – OD pos > 0.7
- % inh positive > 30%

IX – INTERPRETING THE RESULTS

Determine each sample's positivity using the scale shown in Table 1.

Table 1	Calculated value	Degree of positivity
	% inh < 20	0
	20 <= % inh < 40	+
	40 <= % inh < 60	++
	60 <= % inh < 80	+++
	80 <= % inh	++++

X – ORDERING INFORMATION

Monoscreen AbELISA *Clostridium perfringens* epsilon toxin	1 X 96 tests	BIO K 222/1
	2 X 96 tests	BIO K 222/2