Instructions for use
BIOK266-Theta_Ag_NO_(EN)_V04
20/09/2022

MONOSCREEN Ag ELISA

Monoscreen AgELISA *Clostridium perfringens* Theta toxin

Reference : BIO K 266

ELISA kit for detection of Theta toxin of *Clostridium perfringens*

Biwell, sandwich

In vitro and strictly veterinary use

Sample	All species
Culture supernatants	✓
Biological fluids	✓

Presentation

Product reference	BIO K 266/2
Format	2 plates, strip of 8 wells
Reactions	96 tests

Composition of the kit

Provided material	BIO K 266/2
Microplate	2
Washing solution (20X)	1 X 100 mL
Colored dilution solution (1X)	2 X 100 mL
Conjugate (1X)	1 X 25 mL
Positive control (1X)	1 X 4 mL
Single component TMB (1X)	1 X 25 mL
Stop solution (1X)	1 X 15 mL

Revision history

Date	Version	Modifications
20/09/2022	V04	Layout and simplification of the entire leaflet Replacement of the designation “alpha-toxin” by “theta-toxin” Modification of the kit composition : 5X dilution solution is replaced by 1X dilution solution

Note : minor changes to typography, grammar and formatting are not included in the revision history

A. Introduction

Most animal diseases due to *Clostridium perfringens* are intestinal and involve types B, C or D. Type A has been implicated in rare outbreaks of gastritis and haemolytic disease of ruminants (enterotoxemic jaundice, the yellows, yellow lamb disease) and in hemorrhagic enteritis in cattle, horses, dogs and infant alpacas. *Clostridium perfringens* type A causes necrotic enteritis in poultry and a mild form of food poisoning in humans. Demonstration of **Alpha* toxin in the contents of the small intestine is the only way to definitively diagnose enterotoxemia. For that purpose, small amounts of clarified fluid are injected into the tail vein of mice. Death after more than a few minutes postinjection constitues presumptive evidence of enterotoxemia. Other toxins produced by *Clostridium perfringens* have to be neutralized by specific antisera. By using ELISA method, it is possible to detect Alpha toxin in biological fluids (intestinal, peritoneal or pericardic fluid) or in culture supernatants in less than 3 hours. The test can be used to type an unknown strain in conjunction with beta and epsilon Elisa test kits.

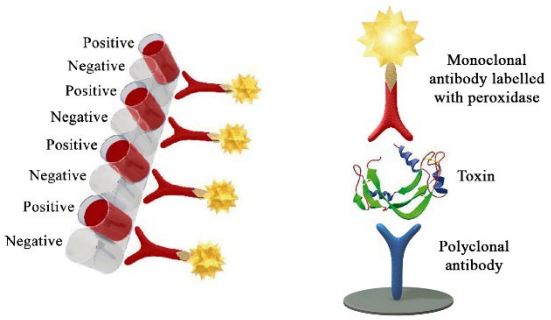
**Recent mass spectrometry analyses have allowed us to demonstrate that the monoclonal antibody used in this kit is not specific for alpha toxin but for theta toxin and a protease.*

As theta toxin is almost always associated with alpha toxin, the purpose of this diagnostic kit for enterotoxemia is not affected by this analytical advance. Moreover, theta toxin is less sensitive to the effect of proteases than alpha toxin and is therefore a more sensitive marker of Clostridium perfringens.

B. Test principle

The test uses 96-well microtitration plates sensitised by specific polyclonal antibodies for the Alpha-toxin. These antibodies allow a specific capture of the corresponding antigen which is present in the samples. *Odd* rows A, C, E, G have been sensitized with these antibodies and *even* rows B, D, F, H are containing nonspecific antibodies. These control rows allow the differentiation between specific immunological reaction and nonspecific 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 dilution. After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate - a peroxidase labelled anti-Alpha-toxin specific monoclonal antibody. After this second incubation, the plate is washed again, and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantage of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If Alpha-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 Alpha-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	Alpha	Thêta	Beta	Epsilon	Iota
A	++	++	-	-	-
B	+	++	++	+	-
C	+	++	++	-	-
D	+	++	-	++	-
E	+	++	-	-	++



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20 µL, 20-200 µL et 100-1000 µL range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer
- Incubator at 37±2°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, ...

D. Precautions for use

- The reagents must be kept between +2 et +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other kits.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1 M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution solution is colored in yellow.
- The conjugates are ready to use.
- The stop solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- The positive control is ready to use.
- Biological samples (intestinal contents, cavity fluids, ...) **must be diluted 2-fold** in the dilution solution.

N.B : The consistency of the sample must be homogeneous. If homogenization is difficult, add glass beads to the container and break up the stool by shaking vigorously. Do not centrifuge.

- Culture supernatants are used **undiluted**.

N.B : The best results have been obtained by using liquid TGY under anaerobic conditions (in a tube without shaking) at 37°C. The optimum culture time for Alpha and Theta toxin is overnight or at least until maximum gas production.

G. Procedure

- Bring all the reagents to **21±3°C** before use.
- Carefully read through the previous points.

N.B. : To avoid differences in incubation time between samples of a large series, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (100 µL) into the test microplate using a multi-channel pipette.

1. Distribute **100 µL per well** of **diluted** biological samples, the positive control of the kit, **ready to use,** and the **undiluted** culture supernatants and a sample or positive control. Biwell column layout (e.g.: sample n°1 : well A1 and B1, positive control: well C1 and D1). Cover with a lid and incubate the plate **at 21 ± 3°C** during **60 ± 5 min**.
2. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.
3. Add **100 µL ready to use conjugate** per well. Cover with a lid and incubate the plate at **21 ± 3°C** during **60 ± 5 min**.
4. Remove the content of the microplate. **Wash the microplate 3 times** with **300 µL** of washing solution per well. Avoid the formation of bubbles in the well between each wash.
5. Distribute **100 µL** of TMB solution per well. Incuber à **21 ± 3°C** pendant **10 ± 1 min** à l'abri de la lumière, sans couvrir.
6. Distribute the stop solution at rate of **50 µL** per well. The colour changes from blue to yellow.
7. Record the optical densities using a plate spectrophotometer with a 450 nm filter **within 5 minutes** after adding the stop solution.

H. Validation of results

The test can only be validated if :

- The test can only be **validated** if the difference between optical density readings (OD) of the odd and even line of the positive control is greater than the value on the QC data sheet included in the kit.

+ Control / theta-toxin : $OD_{\text{odd line}} - OD_{\text{even line}} > QC$

I. Interpretation of results

Calculate for each sample its coefficient (S/P %) using the following formula :

$$S/P\,(\%) = \frac{OD\ odd\ line\ sample - OD\ even\ line\ sample}{OD\ pos.Ctl.\ odd\ line - OD\ pos.Ctl.\ even\ line}$$

With pos. Ctl. = positive control

	Results	Status
Sample	S/P % < x*%	Negative
	S/P % ≥ x*%	Positive

*Determine samples' status using the table in the quality procedure (QC) included in the kit.

Get the interpretation of your results quickly and easily using **AnalysiScreen**, our free online platform, available on our website : https://www.biox.com

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysiScreen™** is :

- Free
- Accessible online via our website: https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

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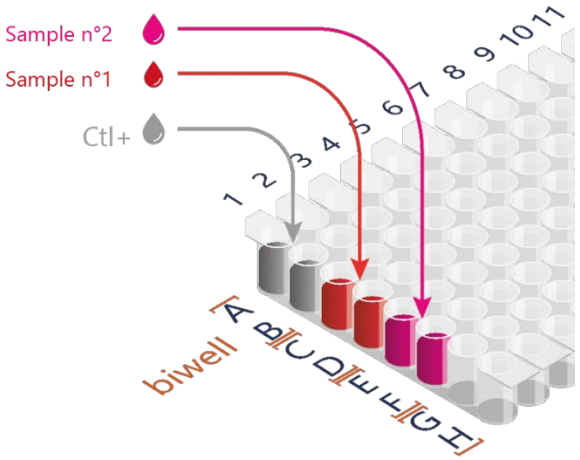
Notes*

1 Distribute 100 µL :
- Dilution of biological samples 1/2
- Culture supernatants 1/1
- Positive control (+Ctl) 1/1

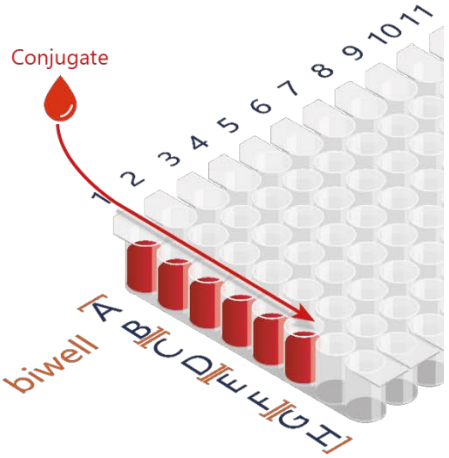
Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of conjugate

Room Temp. — 60 min → 3X Wash



Kit microplate

3 Add 100 µL of TMB

Room Temp. — 10 min — Obscurity

4 Add 50 µL of stop solution

5 Record optical densities

450 nm

* Notes do not replace the instructions for use of which they are a synthesis.

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