Instructions for use
BIOK289-Alpha_Ag_NO_(EN)_V04
28/03/2023

MONOSCREEN Ag ELISA

Monoscreen AgELISA *Clostridium perfringens* Alpha toxin

Reference : BIO K 289

ELISA kit for detection of Alpha toxin of *Clostridium perfringens*
Biwell, sandwich

In vitro and strictly veterinary use

Sample / Dilution	All species
Culture supernatants / 1X	✓
Biological samples / 2X	✓

Presentation

Product reference	BIO K 289/1
Format	1 plate, strip of 8 wells
Reactions	48 tests

Composition of the kit

Provided material	BIO K 289/1
Microplate	1
Washing solution (20X)	1 X 100 mL
Colored dilution solution (1X)	1 X 100 mL
Conjugate (1X)	1 X 12 mL
Positive control (1X)	1 X 4 mL
Single component TMB (1X)	1 X 12 mL
Stop solution (1X)	1 X 6 mL

Revision history

Date	Version	Modifications
28/03/2023	V04	Modification of the test principle: polyclonal antibody replaced by monoclonal antibody. Modification of the composition of the kit : freeze-dried positive control replaced by liquid positive control.

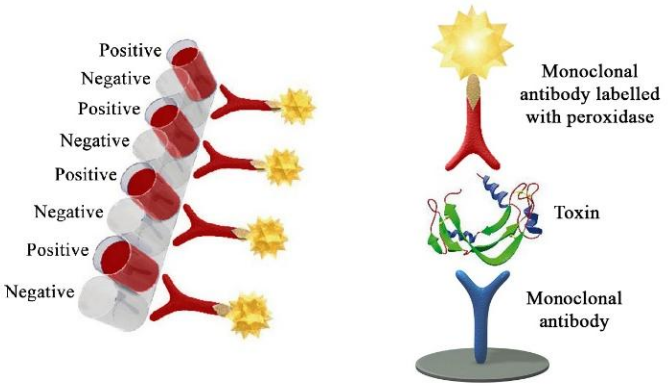
Note : minor changes to typography, grammar and formatting are not included in the revision history

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 constitutes 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 samples (intestinal fluids) 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.

B. Test principle

The test uses 96-well microtitration plates sensitised by specific monoclonal 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: intestine contents) 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 subtracted from the corresponding positive microwells. There is a positive control supplied with the kit.



Toxinotypes	Alpha	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 21±+3°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 conjugate is 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) **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 mixing. Do not centrifuge.

- Culture supernatants are used **undiluted**.

N.B : For optimum detection, we recommend performing an 8h culture at 37°C in TGY medium under anaerobic conditions (e.g. in a 10 ml tube of culture medium, tightly closed, without shaking). After 8h incubation, freeze the culture until use.

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, and the **undiluted** culture supernatants. Biwell vertical layout (e.g.: sample n°1 : well A1 and B1, positive control: well C1 and D1). Cover 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 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 / alpha-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

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
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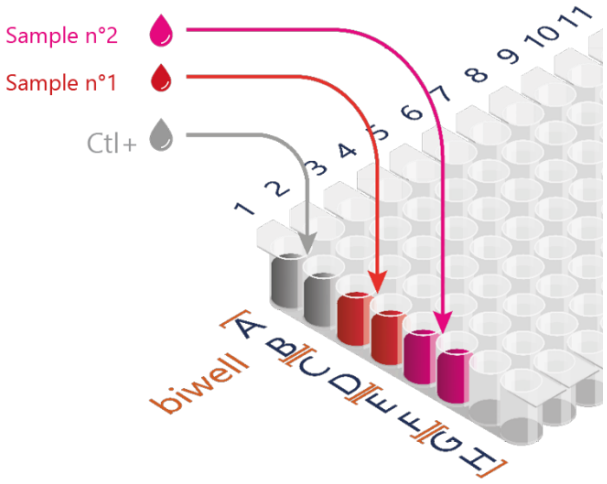
- Very easy to use

SCAN ME

Notes*

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

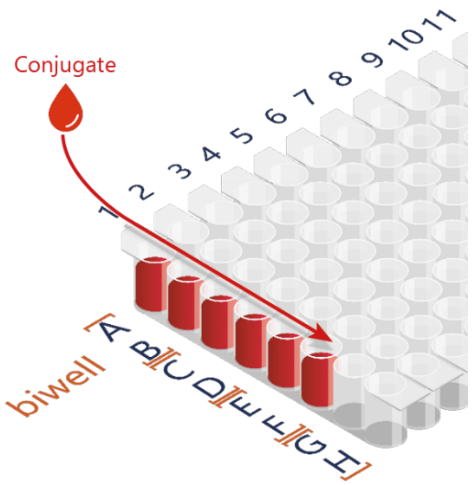
Room Temp. — 60 min → 3X Wash



Kit microplate

2 Add 100 µL of conjugate

Room Temp. — 60 min → 3X Wash



Kit microplate

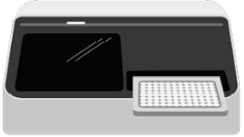
3 Add 100 µL of TMB

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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