tgcBIOMICS
Targeted Genetic Conditioning

Short Instruction Manual of the Assay

ELISA for the detection of *Clostridium difficile* toxin A (or B) antibodies

The ELISA was developed to routinely detect the antibody level against *Clostridium difficile* toxin A or B (TcdA or TcdB) in human sera/plasma and stool specimen. Measurement of serially diluted sera will be an indicator of the strength of the immune response. Our standard test includes the reagents for the detection of human IgG and IgA antibodies against TcdA (or TcdB). The assay is

-for research use only-

The assay is also available as a kit for the detection of the following species- or subtype-specific antibodies

a.	Human	subtype: IgM, IgG, IgA
b.	Hamster	subtype: IgG
c.	Mouse	subtype: IgG, IgA
d.	Bovine	subtype: IgG, IgA

tgcBIOMICS will compose the kit components according to the customers demand (antibody specificity, species and subtypes to be detected).

I. Kit components: the detailed list of the components is attached

All componets should be stored at 4 °C

1. **ELISA plate** - coated with *Clostridium difficile* toxin A **OR** toxin B
2. **Positive control** - species antibody against *Clostridium difficile* toxin A (or B) -ready to use
3. **Conjugate** - peroxidase conjugated anti-species specific antibody - ready to use
4. **Dilution buffer** – 50 ml - ready to use
5. **10x Wash buffer** – 50 ml
6. **TMB–substrate** – 14 ml - ready to use
7. **Stop solution** - 7,5 ml - ready to use

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II. To be prepared prior to testing:

INITIAL CONTROL STEP

- Correct toxin coated plate taken (TcdA OR TcdB)?
- Does the positive control correspond to the plate?
- Appropriate Ig M / G / A Peroxidase conjugate chosen?

1. Prepare 1x wash buffer: the wash buffer is supplied as a 10x concentrate. The 50 ml supplied need to be diluted to a total volume of 500 ml by adding 450 ml aqua dest.. Store your diluted 1x wash buffer between 2 °C and 8 °C to avoid growth of contaminating microbes. Preparation of aliquots of the wash buffer is done accordingly.

2. Microtitre plate preparation: the plates are sealed in aluminium bags and once opened should be resealed with the snap closing. Once opened stability of the plate at 4 °C is about 4-6 months. The plates can be used as broken "single wells" or as single strips.
   Each strip contains 8 wells coated with *Clostridium difficile* toxin A (or B). Prior to testing you need to determine the number of wells for your assaying.
   Do not contaminate the wells with your fingers. Assay wells not used should immediately be returned to the bag and carefully resealed with desiccant.

III. Preparing the samples

Sera / plasma or culture supernatant:
Starting from a 1:100 dilution of the specimen in dilution buffer, we strongly recommend to make further 1:2 dilutions in dilution buffer
(see also section VII).

Stool sample:
Transfer about 50 µl liquid stool sample or take an equivalent amount
(50 mg) of compact stool in 450 µl dilution buffer, homogenize the suspension by suction and ejection from a disposable pipette or by vortexing. After leaving for a short time to allow sedimentation of stool particles the clarified supernatant can be used directly in the test.
Automated equipment may be used with specimen that have been centrifuged 5 min by 2500 x g to remove any particulate matter.

IV. Test procedure

Warnings and precautions:
All reagents and materials which come into contact with potentially infectious samples must be treated with suitable disinfectant or autoclaved; suitable disposable gloves must be worn during the entire test.

All reagents should be at room temperature prior to their use in the assay.

1. Select the ELISA plate of your choice (coated with TcdA or TcdB).

2. Transfer 100 µl of a 1:100 prediluted sample (specimen diluted in dilution buffer) into each single well. As positive control use 100 µl of the supplied control. For the negative control use 100 µl of the dilution buffer.

3. Incubate for 60 min at 37 °C.

4. Discard the contents of the microwells into a collection pan.

5. Wash each well 3 x with wash buffer.
   After washing, completely remove any residual liquid by striking the plate (wells) onto a dry paper.

6. Pipette 100 µl of the conjugate to detect antibodies against toxin A (or B)

7. Incubate for 30 min at 37 °C.

8. Wash each well 3 x with wash buffer.
   After washing, completely remove any residual liquid by striking the plate (wells) onto a dry paper.

9. Thereafter add 100 µl TMB-substrate to each well.

10. Incubate for 15 min at RT.

11. The color development will be stopped by adding 50 µl of the stop solution to each well.

12. Measurement of the extinction will be done with a microtiter plate photometer at 450 nm versus 620 nm.

V. Standard configuration of an assay:

- For internal quality control, negative and positive standards should be run in parallel to each experiment.
- To avoid background reactions predilution of the sera in the range of 1:100 (followed by further dilutions in 2-fold steps) is recommended.

VI. Interpretation of results:

The read out of the assay is based on the measurement of the optical density at
450 nm and 620nm and is calculated as OD_{450}-OD_{620}.

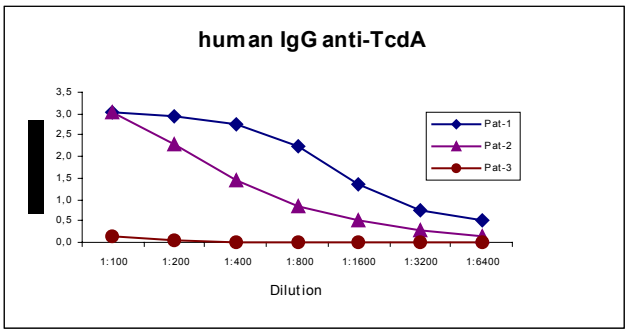
- Negative control:
  The $OD_{450-620}$ background should be below 0,050.

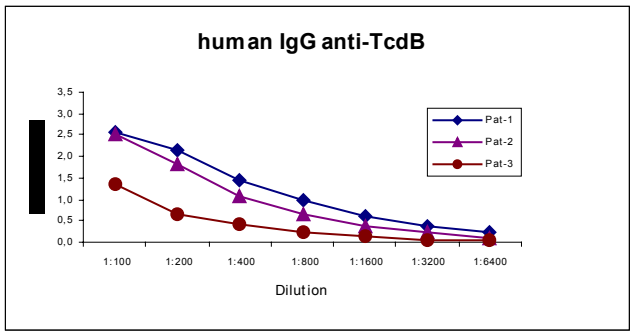
- Positive control:
  The $OD_{450-620}$ of the positive control should be >1,00.

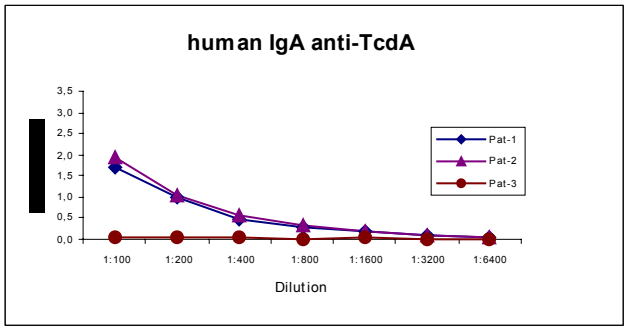
- Cut off value:
  The cut off of the assay is the OD of the negative control + OD 0,100.

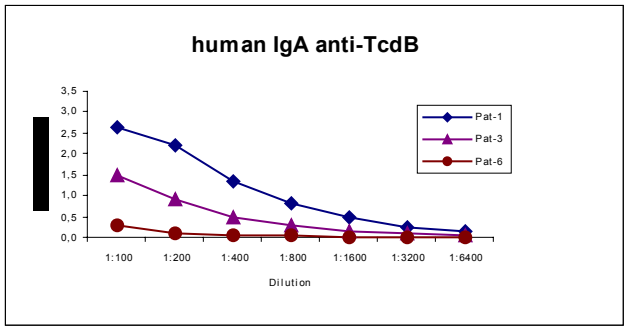
VII. Typical results:

Typical results of an antibody titration of hospitalized patients are given in the attached diagrams. The titration started with a 1:100 pre-dilution of the sera and was done in 2-fold steps.









Pat-1:	**IgG α-TcdA pos; IgA α-TcdA pos**	**IgG α-TcdB pos; IgA α-TcdB pos**
Pat-2:	**IgG α-TcdA pos; IgA α-TcdA pos**	**IgG α-TcdB pos; IgA α-TcdB pos**
Pat-3:	**IgG α-TcdA neg; IgA α-TcdA neg**	**IgG α-TcdB pos; IgA α-TcdB pos**