DRG® E. coli Verotoxin 1+2 Ag ELISA (EIA-4452)

CE

Revised 27 May 2011 rm (Vers. 2.1) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

The ***E. coli Verotoxin 1+2 Ag ELISA*** is a device for direct detection of verotoxin 1 and 2 (shiga-toxin 1 and 2) in faecal specimens and stool culture supernatants.

2 PRINCIPLE OF THE TEST

The *E. coli Verotoxin 1+2 Ag ELISA* is an indirect two-site-immunoassay for the determination of verotoxin 1 and 2 based on polyclonal and monoclonal antibodies.
Verotoxin 1 and/or 2 of specimens and the positive control react with polyclonal anti-verotoxin 1 and 2 antibodies coated on the solid phase of the microplate. After incubation for 60 minutes at 22-25°C non-bound material is removed by a washing step.
Subsequently bound toxins specifically react with biotinylated monoclonal anti-verotoxin 1 and anti-verotoxin 2 antibodies during a second incubation period of 30 min at 22-25°C. Non-bound material is separated from the solid-phase immune complexes by a subsequent washing step.
During the next incubation period of 30 min at 22-25°C horseradish peroxidase (HRP) conjugated streptavidin reacts with the bound biotinylated antibodies. Unbound conjugate is removed by a washing step.
HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the wells after 15 min incubation at 22-25°C turning the solution from blue to yellow.
The optical density (OD) of the solution read at 450/620 nm is directly proportional to the specifically bound amount of verotoxin 1 and/or verotoxin 2.
Considering the cut-off value results are interpreted as positive or negative.

3 PREPARATION AND STORAGE OF SAMPLES

3.1 Collection and storage

Stool samples should be stored at 2 °C – 8 °C immediately after collection and processed within 72 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided.
In case of direct verotoxin detection from stool suspension in sample diluent, testing immediately after the sample has arrived in the laboratory should be preferred.
Stool specimens for enrichment culture should be transferred to the enrichment broth within 1-2 hours after the sample has arrived.

3.2 Preparation

3.2.1 Sample preparation for direct testing from diluted stool specimens

Quickly thaw frozen stool specimens and mix them well. Samples treated with transport media should also be mixed before testing.
Pipette 500 µl of Verotoxin 1+2 sample diluent into a clean tube.
Transfer 200 mg (diameter about 4-5 mm) or 200 µl stool sample into the tube with the 500 µl of Verotoxin 1+2 sample diluent and mix thoroughly.
Caution: the direct investigation of stool specimens with ELISA without prior enrichment should only serve as screening method for a fast preliminary result. A subsequent additional investigation of the concerning sample after enrichment is absolutely necessary to reach a sufficient sensitivity. **A negative ELISA result does not necessarily exclude an infection with EHEC when stool samples are tested without enrichment culture.**

3.2.2 Sample preparation for testing from enrichment culture

Transfer about 200 mg or 200 µl of stool sample into a tube with 4 ml enrichment broth, e.g. EHEC-Direktmedium (Haipha) or mTSB (Mast) containing 50 ng/ml Mitomycin C and
incubate for 18 to 20 hours at 37°C. If possible, use a shaker during sample incubation.
Subsequently allow floating particles to sediment or if necessary sediment floating particles by a centrifugation step.
Dilute the culture supernatant 1:2 with Verotoxin 1+2 sample diluent and dispense 100 µl diluted culture sample per well.

4 TEST COMPONENTS FOR 96 WELLS

1 **WELLS**	**Microtitration plate** 12 single breakable 8-well strips (in all 96 wells) coated with polyclonal anti-Verotoxin 1+2 antibodies (sheep)	1 vacuum sealed with desiccant
2 **WASHBUF CONC 10X**	**Wash buffer, 10-fold** for 1000 ml solution	100 ml concentrate white cap
3 **DIL**	**Sample diluent for Verotoxin 1+2**	100 ml ready to use coloured red black cap
4 **CONTROL +**	**Positive control** Inactivated Verotoxin positive culture supernatant	2.0 ml ready to use coloured blue red cap

5 **CONTROL -**	**Negative control** Verotoxin negative sample	2.0 ml ready to use coloured blue green cap
6/1 **CONJ BIOTIN**	**Biotin-conjugate** Biotinylated, monoclonal anti-Verotoxin 1 and 2 antibodies (mouse)	15 ml ready to use coloured green white cap
6/2 **CONJ STREPT**	**Streptavidin-poly-HRP-conjugate**	15 ml ready to use coloured red brown cap
7 **SUBSTR TMB**	**Substrate** 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide	15 ml ready to use blue cap
8 **STOP**	**Stop solution** 0.25 M Sulphuric acid	15 ml ready to use yellow cap

5 MATERIALS REQUIRED BUT NOT PROVIDED

- adjustable one-channel micropipettes and pipette tips
- adjustable multi-channel pipette or multi-pipette and pipette tips
- Reagent container for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with 450 nm filter for measurement and ≥ 620 nm for reference
- distilled or de-ionized water
- glassware
- tubes (1 ml) for sample preparation
- enrichment broth, e. g. EHEC-Direktmedium (Haipha) or mTSB (Mast) containing 50 ng/ml Mitomycin C

6 PREPARATION AND STORAGE OF REAGENTS

6.1 Kit size and expiry

One kit is designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the outer box label.
Upon receipt, all test components have to be kept at 2-8°C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.
The ready to use wash buffer solution is stable for at least 1 month when stored at 2 °C – 8 °C.

6.2 Reagent preparation

Allow all components to reach room temperature prior to use in the assay.

The microtitration plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed plate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated *Wash Buffer* 10 times (1 + 9) with distilled or de-ionized water.
For Example: 10 ml *WASHBUF CONC 10X* concentrate + 90 ml distilled water.

7 ASSAY PROCEDURE

- Dilute stool samples with Verotoxin 1+2 sample diluent (3) **1 + 2.5**
  e.g. 200 mg or 200 µl stool + 0.5 ml Verotoxin 1+2 sample diluent (3)
- For enrichment culture:
  Transfer 200 mg or 200 µl sample to 4 ml enrichment broth (e.g. EHEC-Direktmedium or mTSB + 50 ng/ml Mitomycin C) and incubate for 18 – 20 hours at 37 °C if possible on a shaker.
- Dilute culture supernatant **1:2** with Verotoxin 1+2 sample diluent, mix thoroughly and use 100 µl/well for ELISA testing.
- Avoid any time shift during dispensing of reagents and samples.
- Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every single wash cycle!
- Avoid light exposure of the TMB substrate solution!

7.1 Working steps

1. Warm all reagents to room temperature (20 °C – 25 °C) before use. Mix gently without causing foam.
2. Dispense:
   3 drops (or 120 µl) ***CONTROL + (4)***
   3 drops (or 120 µl) ***CONTROL - (5)***
   100 µl diluted **specimen** or diluted **culture supernatant**
3. Cover plate and incubate for **60 min** at 22-25°C.
4. Decant, then wash wells **5x** with **300 µl** wash solution (diluted from 2).
5. Dispense **3 drops (or 120 µl)** ***CONJ BIOTIN (6/1)*** per well
6. Cover plate and incubate for **30 min** at 22-25°C.
7. Decant, then wash wells **5x** with **300 µl** wash solution (diluted from 2).

8. Dispense **3 drops (or 120 µl)** ***CONJ STREPT (6/2)*** per well.
9. Cover plate and incubate for **30 min** at 22-25°C.
10. Decant, then wash wells **5x** with **300 µl** wash solution (diluted from 2).
11. Dispense **3 drops (or 120 µl)** ***TMB SUBSTR TMB (7)*** per well.
12. Incubate for **15 min** at 22-25°C protected from light.
13. Dispense **3 drops (or 120 µl)** ***STOP (8),*** mix gently.
14. Read OD at **450 nm** (reference filter ≥ 620 nm) with a microplate reader within 30 min after reaction stop.

7.2 Automatic Processing

Performing the Verotoxin 1+2 ELISA on fully automated microplate processors (e.g. DS2, DSX or PersonalLab) may cause elevated absorbances in comparison to the manual procedure due to individual differences concerning wash procedures and general technical specifications of the equipment. In these cases a maximum value of 0.3 absorbance units is permissible for the negative control.
It is recommended to use a wash procedure including 10 seconds soak time per strip and wash step followed by a wash step with distilled or deionized water with 10 seconds of soak time after the final wash step of each wash cycle.

Correlation: Manual – automatic processing

A panel of 188 specimens was investigated in parallel by manual and automatic processing method resp. The correlation was calculated with r = 0.98.

8 COMMON ADVICES AND PRECAUTIONS

Follow the working instructions carefully. The kit should be performed by trained technical staff only.
The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
Do not use or mix reagents from different lots except for sample diluent, wash buffer, TMB/substrate solution and stop solution.
Do not use reagents from other manufacturers.
Avoid time shift during dispensing of reagents.
All reagents should be kept at 2-8°C before use.
Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.
Handle all components and all samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:
- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.

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