# *E. COLI* ANTISERA

for *in vitro* diagnostic use

**Application**
The *E. coli* diagnostic antisera are intended for complete or partial serotyping by agglutination.

**Description**
The *E. coli* antisera are raised in rabbits and consist of O, OK O and H antisera. The antisera may be used separately or in combination depending on the aim of the test. The antisera are supplied 3 ml (O, OK O and H pools) or 5 ml (H single) bottles (sodium azide as preservation). Cross-reactions have been removed by absorption when necessary.

**Principle**
When a bacterial culture is mixed with a specific antiserum directed against bacterial surface components, the cells are bound together through antigen-antibody bonds to form aggregates (agglutination). This is usually visible to the naked eye as clumps in the suspension. By mixing specific antisera with an *E. coli* culture, the O- and H antigens are determined.

**Material required but not provided**
Agar medium (e.g. beef extract agar), semi solid agar and infusion broth
Inoculating loop or toothpick
Pipettes
Glass tubes (Widal tubes)/microtiterplates or glass slides
Physiological saline, pH 7.4
A water bath or an incubator set at 50-52°C
An incubator set at 37°C
A water bath >90°C

**Procedure**

**General**
Physiological saline is used as a negative control and must be negative. If the negative control is positive (agglutinates), the strain is auto agglutinating, i.e. O rough.

**Slide agglutination with OK O antisera**

1. The *E. coli* is grown over night on a suitable agar medium not inhibiting motility.
2. Apply a small drop of antiserum (approximately 20 µl) on a glass slide.
3. Transfer culture from a single colony to each drop of antiserum and mix well. The amount of culture should be sufficient to give a distinct milky turbidity. Use an inoculating loop or a toothpick.
4. Tilt the slide for **5 - 10 seconds**.
5. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).
6. A positive reaction is seen as a visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination should be considered negative.

It is important to have the O group confirmed by agglutination with O antisera using a boiled culture (see below).

**O-agglutination of boiled culture with O antisera**
One colony on an agar medium is selected for inoculation in infusion broth and incubated overnight at 37°C. Boil the broth culture ≥90°C for 1 hour. The freshly boiled culture must always be left on the table for 1 hour before use in order to allow sedimentation of bacterial debris. Boiled cultures can be stored at 4°C but must be turned upside down a few times and left for 1 hour on the table before use.
High titre O antiserum must be diluted before use. Recommended working dilution is printed on the label.

1. Mix 3 drops of O antiserum (approximately 180 µl) for glass tubes or 80 µl for microtiterplates with an aliquot amount of boiled culture.
2. The glass tubes or microtiterplates is incubated in a humid atmosphere at 50-52°C overnight.
3. The reaction is read against artificial light with a black background after vigorous shaking of the glass tubes. The microtiterplates must **not** be shaken.
4. In glass tubes a positive reaction is seen as a granular reaction. A negative reaction is persistence of the homogeneous milky turbidity. In microtiterplates a positive reaction is seen as a "grey carpet", covering the bottom of the well. When the reaction is negative the bacterial suspension is a small white spot centred in the well.

**H-agglutination of formalin killed culture with H-antisera**
One colony on an agar medium is selected for inoculation on a semi solid agar and incubated overnight at 37°C. Pass the motile culture t infusion broth with a Pasteur pipette, incubate for 6 hours at 37°C a fix with a formaldehyde concentration of 0.5%.
The H antiserum is ready-to-use.

The agglutination is easily seen in tubes. Microtiterplates are only recommended for very rutined persons, because it is difficult to distinguish between O and H agglutination.

1. Mix 3 drops H antiserum (approximately 180 µl) for glass tubes or 100 µl for microtiterplates with an aliquot amount of formaldehyde fixed culture.
2. The glass tubes or microtiterplates are incubated in a humid atmosphere at 50-52°C for 1½ to 2 hours (maximum). **Do not shake or rock the tubes when removing them.**
3. After a very gentle tilt of the tubes they are read against artificial light with a black background (indirect illumination).
4. In glass tubes the typical H agglutinates are loose and fluffy but, like agglutinates of other surface antigens, can be strongly influenced in shape and structure by the other antigens present. In microtiterplates the reaction looks like the O agglutination but the H agglutination looks like 1-2 mm strings, while the O agglutination looks like dots.

**Storage and shelf life**
Store at 2-8°C in a dark place. Expiry date is printed on the package. Turbidity due to lipoprotein precipitation is sometimes seen after prolonged storage. Precipitation and/or contamination can be removed by centrifugation (10,000g) followed by sterile filtration (0.22 µm).

**Typing support**
*E. coli* strains which cannot be typed, may be sent to The International Escherichia Centre (WHO), 5 Artillerivej, 2300 Copenhagen S, Denmark for further examination. Please enquire about the charges.



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