Application
The E. coli K9 antiserum for slide agglutination from Statens Serum Institut is intended for screening of E. coli O104 strains. It is important to confirm the result with an O104 antiserum for use with boiled culture.

Description
The E. coli antiserum is raised in rabbits. The antiserum is supplied in 1 mL bottles (sodium azide as preservation).

Principle
When a bacterial culture is mixed with the corresponding specific antiserum directed against the bacterial surface components, the bacteria 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.

Material required but not provided
Agar medium (e.g. beef extract agar)
Inoculating loop or toothpick
Physiological saline, pH 7.4
An incubator set at 37°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.

Slide agglutination with K9 antiserum
The E. coli is grown over night at 37°C on a suitable agar medium not inhibiting motility.

1. Apply a small drop of antiserum (approximately 20 μL) on a glass slide.
2. Transfer culture from a single colony to the drop of antiserum and mix well.
3. Tilt the slide forth and back for no more than 5 - 10 seconds.
4. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).
5. 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.
6. A positive result should be confirmed by a positive O104 antiserum reaction.

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), S Ørestad Boulevard, DK-2300 Copenhagen S, Denmark for further examination. Please enquire about the charges.

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