

Toxigenic *Escherichia coli* Pili Antisera "SEIKEN"

Toxigenic *Escherichia coli* possesses special pili which are antigenetically different from those of other *E. coli*.

The pili are related not only to attachment and infection of the organism to intestinal mucous epithelial cells but also to its ability to produce enterotoxin.

It is possible to screen toxigenic *E. coli* using pili antisera because specific adhesive pili antigens are observed only on toxigenic *E. coli*.

The products are immune sera prepared by hyperimmunizing healthy rabbits with adhesive pili antigen purified from toxigenic *E. coli* of domestic animals and contains sodium azide at a concentration of 0.1 w/v% as a preservative.

PRODUCT

5 ml × 3 vials per set: K88, K99, 987P

INTENDED USE

The reagent is used for serotyping *E. coli* pili antigen.

PRINCIPLE

When this reagent is mixed with the homologous *E. coli* organisms an antigen antibody reaction occurs and forms an agglutinate of cells visible with the naked eye. Thus, serotyping is carried out.

PROCEDURES

1. Incubate the isolated cells using Minca medium at 37°C for 18 hours.
2. Prepare a bacterial cell suspension by densely suspending the cells in physiological saline.
3. Using a glass pencil, divide a glass slide into several sections, and place one drop of each serum and physiological saline (control) onto each section.
4. Place one drop of test bacterium suspension on the slide and mix it well.
5. Only obvious agglutination occurring after one minute should be taken as positive. Spontaneous antigen agglutination should be checked using physiological saline as a control.

Formulation of Minca medium

KH ₂ PO ₄	1.36 g
*Na ₂ HPO ₄ •2H ₂ O	10.1 g
Glucose	1 g
**Casein acid hydrolysate	1 g
Yeast extract	1.5 g
Agar-agar	12 g
***Mineral solution	1 ml
Distilled water	1000 ml
pH 7.5	

* If Na₂HPO₄•12H₂O is used instead of Na₂HPO₄•2H₂O: 20.3 g

** Casamino-Acids (Difco)

*** Formulation of mineral solution

MgSO ₄ •7H ₂ O	10 g
MnCl ₂ •4H ₂ O	1 g

FeCl₃•6H₂O 0.135 g

CaCl₂•2H₂O 0.4 g

Dissolve the above reagents in 1000 ml distilled water.

PRECAUTIONS

1. Adhesive pili antigen of human origin toxigenic *E. coli* does not show agglutination with this reagent.
2. Addition of Iso Vitale X (BBL) at 1 w/v% to the Minca medium will produce stronger reactions with this reagent because it promotes the growth of pili antigen.³⁾
3. Correct selection of colonies is important because the strains possessing 987P antigen also produce non-piliated colonies on the Minca medium.³⁾
4. When the 987P antigen is identified, the addition of ammonium sulphate at a concentration of 2 g/l instead of casein acid hydrolysate to Minca medium will produce stronger reactions.
5. All live cells, glass slides and test tubes used in this test should be sterilized by either soaking in 0.1% sodium hypochlorite solution for more than 1 hour or by autoclaving at 121 °C for more than 20 minutes.
6. If the serum is frozen, it may produce a sediment when thawed. Take care not to freeze the antisera.
7. This reagent contains 0.1 w/v% sodium azide as a preservative. Sodium azide may react with lead and copper pipes to form highly explosive metal azides. Upon disposal, flush away with a large volume of water to prevent azide accumulation.

STORAGE AND SHELF LIFE

Storage temperature: 2–10 °C

Shelf life: Up to expiry date on the label.

PACKAGES

Each type in a 5 ml vial with a syringe

3 vials per set: K88, K99, 987P

REFERENCES

1. Guinee, P. A. M. et al.: Detection of the K99 antigen by means of agglutination and immunoelectrophoresis in *Escherichia coli* isolates from calves and its correlation with enterotoxigenicity, *Infect. Immun.*, **13**, 1369 (1976).
2. Guinee, P. A. M. et al.: Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*, *Infect. Immun.*, **15**, 676 (1977).
3. Nagy, B. et al.: Colonization of porcine intestine by enterotoxigenic *Escherichia coli*: selection of piliated forms In vivo, adhesion of piliated forms to epithelial cells in vitro, and incidence of pilus antigen among porcine enteropathogenic *E. coli*, *Infect. Immun.*, **16**, 344 (1977).