STATENS SERUM INSTITUT

prevention and control of infectious diseases and congenital disorders

5 Artillerivej
2300 Copenhagen S
Denmark

Tel: +45 3268 3268
Fax: +45 3268 3868
serum@ssi.dk
www.ssi.dk

Escherichia coli K-determination with phage cross-brush

For research use

USE

E. coli phage suspensions from Statens Serum Institut is used for determination of K1 and K5 capsule polysaccharide (1).

DESCRIPTION

SSI produces 2 different phage suspensions of K1 and K5 phages.

PRINCIPLE

The method used is called cross-brush:
A dense bacterial culture is streaked across a line of a fresh phage suspension on a nutrient culture medium. Bacteria and phages will mix after the cross and bacteria expressing receptors for the phage will be infected resulting in lysis.
After four hours, the bacterial culture will show significant growth in the streak before the cross with the phage line and no or only poor growth after the line of the phage.
Bacteria not expressing a phage receptor will not be infected and growth will be observed in the full length of the bacterial streak across the phage line.

PRECAUTIONS

General safety procedures should be observed during unpacking and opening of cultures containing *E. coli*. The phage suspensions are intended for use by qualified professionals who are familiar with their use and trained in good laboratory procedures only. Direct contact should be avoided. To prevent contamination the handling of phage suspensions should be aseptically.

INSTRUCTION_ - (see picture)

1st day:

A beef broth culture is inoculated with the selected strains and incubated at 37°C overnight (approximately 10^8 cfu).

2nd day:

1. With a 10 µl inoculation loop a vertical line of phage suspension is applied onto a beef broth agar plate. The phage suspension is applied equally in the whole length of the line by drawing the loop up and down a couple of times. Allow sufficient time for the phage suspension to dry
2. Streak a horizontal line from left to right crossing the phage suspension with approximately 1 µl live broth culture.
3. The agar plate is incubated for 4 hours at 37°C, read and placed at 4°C. The day after, it may be incubated at 37°C for a couple of hours before the final reading.
4. The **positive** reaction is seen by no growth after the cross with the phage line. Positive reaction can also be identified by the plaque method.
   The **negative** reaction is seen by growth in full length of the bacterial streak.
   The maximum for each agar plate is 6 bacterial cultures and 1 positive control strain.
   Positive control strains:

	Original No.	Serotype
K1:	U 9-41	O2:K1:H4
K5:	Bi8337	O10:K5:H4

REFERENCES

1. **Ørskov F, I Ørskov.** 1984. Serotyping of *Escherichia coli*. Methods in Microbiol. **14**:43-112.

LIABILITY

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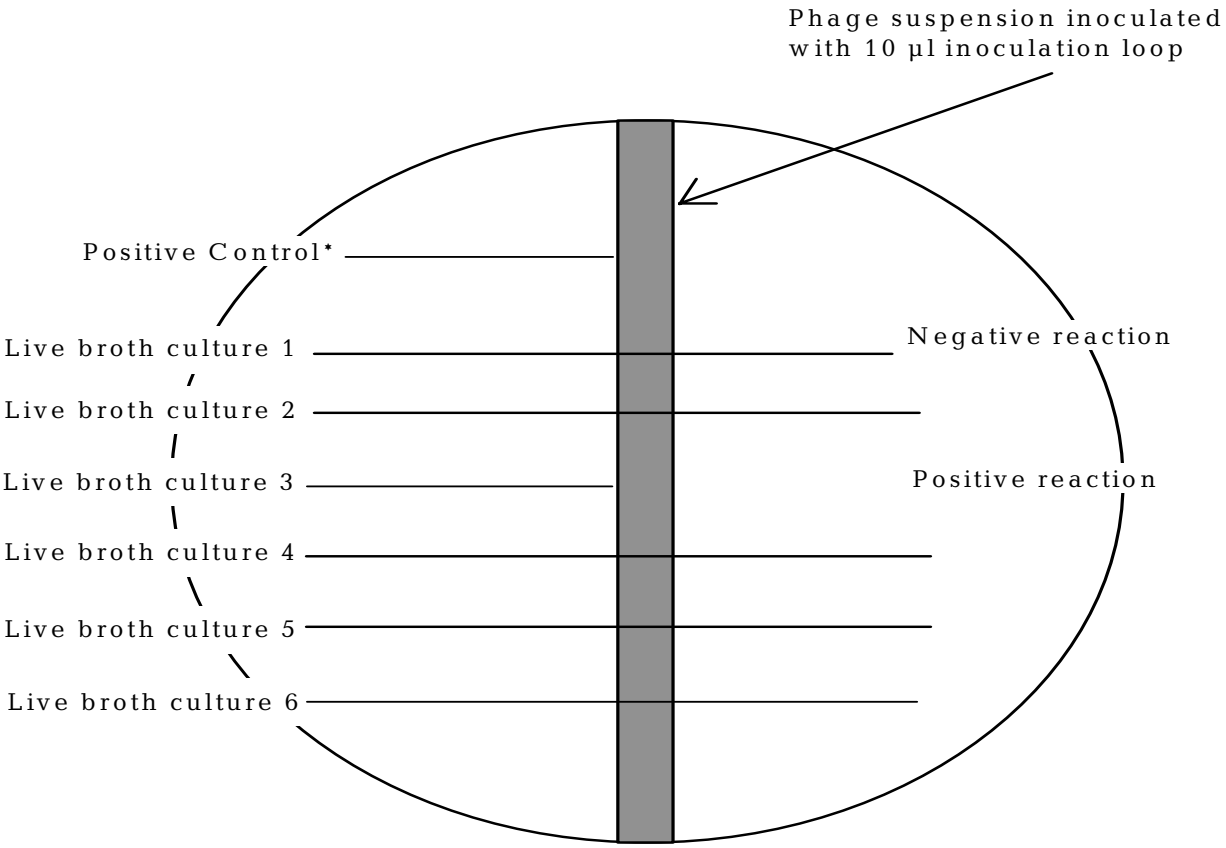

*) **Positive control strains:**

	Originalnr.	Serotypes
K1	U 9-41	O2:K1:H4
K5	Bi8337-41	O10:K5:H4

INFORMATION and ORDERING

Phone +45 32 68 83 78
Fax +45 32 68 81 79
E-mail: microbiology@ssi.dk