Escherichia coli/Sera

Agglutinating sera for diagnosis of somatic antigens of enteropathogenic *E. coli* in infants (EPEC)

PRESENTATION

The following sera are presented in 3 ml dropper bottles. The sera are diluted by agglutination on slides. They do not contain any H agglutinins.

- 1 nonvalent serum **code 355-7411**

- 4 trivalent sera
  Mix I: **code 355-7331**
  O111 + O55 + O26
  Mix II: **code 355-7341**
  O86 + O119 + O127
  Mix III: **code 355-7351**
  O125 + O126 + O128
  Mix IV: **code 355-7361**
  O114 + O124 + O142

- 4 packs, each containing 2 bottles of a trivalent mixture and 3 bottles of corresponding monovalent sera:

- 12 monovalent sera:
  - O111 **code 355-7241**
  - O55 **code 355-7221**
  - O26 **code 355-7211**
  - O86 **code 355 7231**
  - O119 **code 355-7251**
  - O127 **code 355-7281**
  - O125 **code 355-7261**
  - O126 **code 355-7271**
  - O128 **code 355-7291**
  - O124 **code 355-7201**
  - O114 **code 355-7301**
  - O142 **code 355-7311**

DEFINITION

Certain types of *Escherichia coli* can cause diarrhoea. According to the mechanism of their pathogenicity, they are classified into:

- **Enterotoxigenic *E. coli* (ETEC)** causing choleriform diarrhea, frequent in tropical regions with poor sanitary conditions, particularly rampant in children under 5 and in visitors to these countries. In countries with better standards of sanitation, these ETEC are rare, although they can occasionally cause epidemics in nurseries. Their diagnosis is arrived at by detection of the thermolabile enterotoxin (LT) or the thermostabile enterotoxin (ST). Like choleric vibrios, they adhere to the intestinal cells but do not penetrate them.

- **Entero-invasive *E. coli* (EIEC)** causing dysen-teriform syndromes. The mechanism of the pathogenicity of these *E. coli*, which are usually LDC-positive and immobile, is identical to that of *Shigella*. They are detected by testing invasiveness (HeLa cells culture, or by a positive Sereny test carried out by depositing a drop of culture on the eye of a guinea pig).

- **Enteropathogenic *E. coli* (EPEC)** causing epi-demics of diarrhea in communities of children aged under a year, or up to a maximum of 2 years. Epidemics due to these *E. Coli* were frequent up to 1960 in western Europe. Their frequency has considerably diminished since, without the reason for this being understood. Conversely, in other countries they have remained the principal cause of diarrhea in young babies (e.g. O111 et O119 in Brazil). The mechanism of their pathogenicity was unknown until recent years, although the pathogenic role had been demonstrated by epidemiological studies and tests on volunteers. It is now known that they produce a cytotoxin active on Vero cells (whence its name of Vero-toxin or VT), similar to those of *Shigella dysenteriae*. These EPEC adhere to the epithelial cells of the small intestine and destroy the brush-like borders (whence the epithet “fixers-eradicators”).

There is a correlation between the serotype and the pathogenicity of EPEC. To identify a serotype of mobile *E. coli*, its O antigen and its H antigen must be identified and, if it exists, its K antigen. Identification of H antigen requires several days because it is necessary to render the strain highly mobile. K antigens of EPECs were classed as type B by Kaufman, a same B antigen being linked to a same O antigen (e.g. B4 with 0111). The reality of these B antigens was contested by Orskov (Bact. Rev., 1977, 41, 667-720) because it is impossible to prepare an anti-B serum without corresponding O agglutinins. This explains why the classic terminology such as O111: B_4: H_2 has been abandoned and replaced by O111: H_2.

In clinical laboratory practice, for reasons of rapidity, work is limited to identification of the O antigen, despite it being known that several O + H serotypes, of different pathogenic potential, can have the same O specificity in common. This identification of the O antigen is more

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reliable by agglutination and titration in tubes. But agglutination on slides, which gives an immediate result, is widely used in medical bacteriology laboratories because it is rapid, simple, and gives very few erroneous results when performed by an operator with a degree of competence.

Nine O serotypes of EPECs are encountered in western Europe:

O111	**O86**	**O125**
O55	**O119**	**O126**
O26	**O127**	**O128**

In addition, strains of 3 other serogroups, one of which (O124) corresponds to entero-invasive strains, can be encountered less frequently:

- **O124**
- **O114**
- **O142**

Corresponding sera have been prepared by immunizing rabbits with 18-hour cultures of immobile variants (H) killed by formaling.

METHODOLOGY

Isolation of the strain

It is recommended that a Gram staining be carried out on stools. A wide variety of Gram positive and Gram-negative bacteria are found in normal stools. In *E. coli enteritis* in infants, a distinct predominance of Gram-negative bacilli is found. This microscopic examination may permit rapid detection of complications due to *Candida* or to *staphylococci* resistant to antibiotics during a long treatment involving the latter.

- Inoculate the stools onto a medium which does not inhibit *E. coli* (BCP Lactose agar, Drigalski lactose agar, EMB lactose agar).

(See corresponding Technical Sheet(s))

A systematic parallel detection of *Salmonella*, *Shigella* and *Yersinia enterocolitica*, and even of Campylobacter and Rotavirus is also recommended.

In the acute phase of the disease, enteropathogenic *E. coli* in culture are almost pure.

AGGLUTINATION ON SLIDES

It is important to use impeccably clean glass slides. Deposit a drop of serum on the slide. Collect the culture and place it directly in suspension in the drop of serum. Mix well.

Examine with the naked eye against a dark background or, better still, over a concave mirror.

N.B.: All *E. coli* belonging to one of these serogroups give a total massive agglutination, appearing immediately.

In everyday practice, diagnosis is carried out by detection of agglutination of at least 5 colonies, firstly with the nonvalent serum.

If agglutination is observed with this nonvalent serum, the antigen type is then determined using trivalent sera (Mixes I, II, and III.), followed by monovalent sera corresponding to the trivalent serum giving a positive response.

If no agglutination is found in the nonvalent serum, then the same procedure is followed using Mix IV, corresponding to the currently infrequent type. Colonies agglutinated by this serum are studied in the monovalent sera making up this mixture.

N.B.: It sometimes happens that strains are agglutinated by the nonvalent serum alone, or by the nonvalent and the trivalent, but not by the monovalent sera. This non-specific phenomenon is due to the large quantity of proteins contained in the serum mixes. Only typical agglutination in one of the monovalent sera permits diagnosis.

Delayed agglutination, usually thin, is of no diagnostic value. It can be observed with strains that have O antigen fractions in common with enteropathogenic *E. coli*. Similarly, thin agglutination in all sera will be a reason for suspecting the state R of a strain. In this case, the strain is also auto-agglutinable in isotonic saline water.

It is preferable to detect these agglutinations using cultures on agar or lactose media rather than cultures on ordinary nutrient agar.

Common antigen factors exist between serogroups O86 and O127.

These strains are agglutinated strongly, later and with markedly less intensity by homologous serum tha
n by heterologous serum.

AGGLUTINATION IN TUBES

To check agglutination on slides in the event of doubt, tube titration can be used.

The same sera are used, taking their dilution ratio into account: 1/20

We recommend using a 6 – 8 hour culture on broth as antigen:

- one part is used live (B),
- the other part is used after 2 hours heating in a water-bath at 100°C (O).

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These 2 suspensions are diluted 50% in isotonic saline solution, so as to obtain an optical density corresponding to approximately 5.108 bacteria per ml.

For titration of the live bacteria suspension, serum dilutions range from 1/100 to 1/800, and that of the heated suspension from 1/100 to 1/6400.

- Centrifuge for 5 minutes at about 3,000 rpm (the supernatant should be clear).
- Put the pellet back into suspension by tapping the base of the tube:
  - if the suspension becomes homogenous again, the result is negative,
  - if this is not the case, agglutinations visible to the naked eye are observed = the result is positive.

Results

The agglutination titers are:
- for the live suspension: minimum 1/200 and generally 1/400.
- For the heated suspension: minimum 1/600 and generally 1/3200.

STORAGE

- Ready to use: + 2°C to 8°C
- Expiration date and batch number are shown on the package.

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Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Escherichia coli / Sera / Titration / Enterotoxigenic / Entero-invasive / Enteropathogenic /

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