

# ANTISERUM ESCHERICHIA COLI

## SEROTYPING OF ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC)

IVD

### 1- CLINICAL VALUE

Certain types of *Escherichia coli* can cause diarrhoea. They are classified according to the mechanism of their pathogenicity as:

- Enterotoxigenic *E. coli* (ETEC) causing cholera-like diarrhoea. They are diagnosed by demonstrating the heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST).
- Enteroinvasive *E. coli* (EIEC) causing dysentery-like syndromes. They are detected by the invasiveness test (culture of HeLa cells, or positive Sereny test performed by depositing a drop of culture on a guinea-pig's eye).
- **Enteropathogenic *E. coli* (EPEC)** causing diarrhoea epidemics in institutionalized children under the age of one year **to a maximum of 2 years**. They produce a cytotoxin that is active on Vero cells (hence the name of Vero-Toxin or VT) which resembles those secreted by *Shigella dysenteriae*.

These EPEC adhere to epithelial cells of the small intestine and destroy the brush border (described as "attaching/effacing").

There is a correlation between serotype and pathogenicity of EPEC.

### 2- OBJECTIVE OF THE TEST

*Escherichia coli* antisera are used for serological identification of cultures of enteropathogenic *Escherichia coli* by the slide agglutination method.

### 3- PRINCIPLE

The test is based on agglutination, by specific sera, of bacteria possessing the corresponding antigens.

The serotype of a mobile *E. coli* is defined by its O antigen, its H antigen and, when present, its K antigen.

Identification of the H antigen takes several days, as the strain must be made very mobile. It is therefore difficult to perform in routine practice.

To obtain more rapid results, EPEC serotyping is therefore limited, in routine practice, to identification of the O antigen (although several O + H serotypes, with various pathogenicities, can have a common O specificity).

- Nine O serogroups of EPEC are encountered in Western Europe:

|      |      |      |
|------|------|------|
| 0111 | 086  | 0125 |
| 055  | 0119 | 0126 |
| 026  | 0127 | 0128 |

- Three other serogroups (including O124 which corresponds to enteroinvasive strains) are encountered with a lower frequency:

|      |      |      |
|------|------|------|
| 0124 | 0114 | 0142 |
|------|------|------|

These antisera are obtained by immunizing rabbits with 18-hour cultures of formalin-killed immobile variants (H).

### 4- HOW SUPPLIED

Polyvalent sera and monovalent sera are supplied in 3 ml dropper bottles (60 tests).

| Polyvalent sera                                 |                                                                                        |            |                                              |      |            |
|-------------------------------------------------|----------------------------------------------------------------------------------------|------------|----------------------------------------------|------|------------|
| Antiserum <i>Escherichia coli</i> nonavalent    | mixture of 9 serotypes:<br>(0111 + 055 + 026 + 086 + 0119 + 0127 + 0125 + 0126 + 0128) |            |                                              |      | code 57411 |
| Trivalent sera                                  |                                                                                        |            |                                              |      |            |
| Antiserum <i>Escherichia coli</i> trivalent I   | (O111 + O55 + O26)                                                                     |            |                                              |      | code 57331 |
| Antiserum <i>Escherichia coli</i> trivalent II  | (O86 + O119 + O127)                                                                    |            |                                              |      | code 57341 |
| Antiserum <i>Escherichia coli</i> trivalent III | (O125 + O126 + O128)                                                                   |            |                                              |      | code 57351 |
| Antiserum <i>Escherichia coli</i> trivalent IV  | (O114 + O124 + O142)                                                                   |            |                                              |      | code 57361 |
| Monovalent sera                                 |                                                                                        |            |                                              |      |            |
| Antiserum <i>Escherichia coli</i> monovalent    | O124                                                                                   | code 57201 | Antiserum <i>Escherichia coli</i> monovalent | O125 | code 57261 |
| Antiserum <i>Escherichia coli</i> monovalent    | O26                                                                                    | code 57211 | Antiserum <i>Escherichia coli</i> monovalent | O126 | code 57271 |
| Antiserum <i>Escherichia coli</i> monovalent    | O55                                                                                    | code 57221 | Antiserum <i>Escherichia coli</i> monovalent | O127 | code 57281 |
| Antiserum <i>Escherichia coli</i> monovalent    | O86                                                                                    | code 57231 | Antiserum <i>Escherichia coli</i> monovalent | O128 | code 57291 |
| Antiserum <i>Escherichia coli</i> monovalent    | O111                                                                                   | code 57241 | Antiserum <i>Escherichia coli</i> monovalent | O114 | code 57301 |
| Antiserum <i>Escherichia coli</i> monovalent    | O119                                                                                   | code 57251 | Antiserum <i>Escherichia coli</i> monovalent | O142 | code 57311 |

## 5- STORAGE

Sera stored at +2-8°C in the absence of contamination are stable until the expiry date indicated on the kit (even when opened).

## 6- MATERIAL REQUIRED BUT NOT SUPPLIED

- Glass slide.
- Plastic or platinum inoculation loop.
- Physiological saline.

## 7- PRECAUTIONS FOR USE

- Always comply with current techniques and precautions concerning protection against microbiological hazards, for handling and elimination of material and biological products used for the agglutination reaction.
- These sera contain <0.1% sodium azide. Sodium azide can react with lead or copper present in the plumbing forming explosive metallic azides. When eliminating these reagents, rinse abundantly with water to avoid the formation of azide deposits.
- Do not dilute reagents.

## 8- PROCEDURE

Serotyping is performed after identification of the species on a fresh, pure culture of *E. coli* isolated on non-selective agar medium. Perform a control test on the strain to be tested in physiological saline:

- Take one loop of culture.
- Suspend these bacteria in a drop of physiological saline, ensuring homogeneous suspension.

No agglutination should be observed with physiological saline. If agglutination is observed, it correspond to a self-agglutinating strain self and the test with antisera cannot be performed.

- Deposit 1 drop of immune serum on the slide.
- Take one loop of fresh, pure culture of *E. coli*.
- Suspend these bacteria in a drop of physiological saline, ensuring homogeneous suspension gradually adding bacteria to the serum.
- Examine the mixture with the naked eye over a dark surface or over a concave mirror.

## 9- INTERPRETATION OF THE RESULTS

A positive reaction corresponds to the appearance of massive and immediate agglutination. Any agglutination appearing more than **5 seconds** after suspension is disregarded.

## 10-TEST PERFORMANCES/QUALITY CONTROL

The activity of *Escherichia coli* antisera is controlled with the following strains:

|                           |      | Sera |     |     |             |     |      |      |              |      |      |      |               |      |      |      |              |            |
|---------------------------|------|------|-----|-----|-------------|-----|------|------|--------------|------|------|------|---------------|------|------|------|--------------|------------|
|                           |      | O111 | O55 | O26 | Trivalent I | O86 | O119 | O127 | Trivalent II | O125 | O126 | O128 | Trivalent III | O124 | O114 | O142 | Trivalent IV | Nonavalent |
| <i>E. coli</i><br>strains | O111 | +    |     |     | +           |     |      |      |              |      |      |      |               |      |      |      |              | +          |
|                           | O55  |      | +   |     | +           |     |      |      |              |      |      |      |               |      |      |      |              | +          |
|                           | O26  |      |     | +   | +           |     |      |      |              |      |      |      |               |      |      |      |              | +          |
|                           | O86  |      |     |     |             | +   |      |      | +            |      |      |      |               |      |      |      |              | +          |
|                           | O119 |      |     |     |             |     | +    |      | +            |      |      |      |               |      |      |      |              | +          |
|                           | O127 |      |     |     |             |     |      | +    | +            |      |      |      |               |      |      |      |              | +          |
|                           | O125 |      |     |     |             |     |      |      |              | +    |      |      | +             |      |      |      |              | +          |
|                           | O126 |      |     |     |             |     |      |      |              |      | +    |      | +             |      |      |      |              | +          |
|                           | O128 |      |     |     |             |     |      |      |              |      |      | +    | +             |      |      |      |              | +          |
|                           | O124 |      |     |     |             |     |      |      |              |      |      |      |               | +    |      |      | +            |            |
|                           | O114 |      |     |     |             |     |      |      |              |      |      |      |               |      | +    |      | +            |            |
|                           | O142 |      |     |     |             |     |      |      |              |      |      |      |               |      |      | +    | +            |            |

+ : positive reaction → instantaneous agglutination

## 11-QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

## 12-LIMITS OF USE

- Identification of the bacterial species must be performed before determination of the serogroup.
- Some strains may be agglutinated only by the nonavalent serum, or by the nonavalent and trivalent sera, but are not agglutinated by monovalent sera. This nonspecific phenomenon is due to the large quantity of protein present in polyvalent sera. Identification can only be based on specific agglutination with one of the monovalent sera.
- Delayed agglutination, generally fine, does not have any diagnostic value. It can be observed with strains possessing O antigen fractions in common with enteropathogenic *E. coli*. Similarly, fine agglutination in all sera is suggestive of the R status of the strain. These strains are also self-agglutinating in physiological saline.
- It is preferable to test for these agglutinations by using cultures on glucose or lactose medium rather than cultures on standard nutrient agar.
- There are common antigenic factors between serogroups O86 and O127. These strains are therefore strongly agglutinated by the homologous serum, and later, with a much lower intensity, by the heterologous serum.



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