Escherichia coli Agglutinating Sera

**1. INTENDED USE**

*Escherichia coli* Agglutinating Sera are intended for use in agglutination tests for the presumptive identification of *E. coli* serotypes traditionally associated with infantile gastroenteritis. However, since antigenic components are shared widely throughout the Enterobacteriaceae it is important to confirm by biochemical tests that an organism is of the species *E. coli* when attempting serological identification.

**2. SUMMARY AND EXPLANATION OF THE TEST**

Most pathogenic *Escherichia coli* strains from infantile gastroenteritis have the classical biochemical reactions of the faecal type as shown in Table 2.1. (Some strains of *E. coli* may be encountered which are non-lactose fermenting, anaerogenic, or indole negative, and these should not be excluded from serological analysis.)

**Table 1**

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Inositol</th>
<th>Inulin</th>
<th>Adonitol</th>
<th>Cellobiose</th>
<th>Indole</th>
<th>H₂S</th>
<th>Urease</th>
<th>Simmonds</th>
<th>Citrate</th>
<th>Methyl Red</th>
<th>Voges–Proskauer</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS**

See also Warnings and Precautions

**CAUTION**

- The sera should be stored at 2 to 8°C under which condition they will retain their potency at least until the date shown on the bottle label.

**E. coli Agglutinating Sera**

Produced in rabbits and preserved with 0.5% phenol. Sera are supplied in bottles fitted with tear and dropper. Each bottle contains 2 ml liquid and is supplied ready for use.

On storage some sera become slightly turbid. This does not necessarily indicate deterioration and normally it will not interfere with the results, but the sera may be clarified by centrifugation or membrane filtration (0.45 µm) before use. Gross turbidity indicates contamination and such sera should be discarded.

**3. PRINCIPLE OF THE PROCEDURE**

Serological tests are based on the fact that antibodies in serum, produced in response to exposure to bacterial antigens, will agglutinate with bacteria carrying homologous antigens.

It is advisable to test cultures first with polyvalent sera, which are intended for use by the slide agglutination technique only. Both the confluent growth and selected colonies from the primary plate should be examined. Once the presence of pathogenic types has been indicated by the use of polyvalent sera, further identification can be made with the monovalent sera. These are suitable for both slide and tube agglutination tests. A positive slide reaction with a live culture may be due to the presence of K antigen on the surface of the organisms and the O group should be confirmed by tube agglutination using an O suspension, prepared by heating a suspension of the live culture for one hour at 100°C.

**4. REAGENTS**

**KIT CONTENTS**

- 1 dropper bottle (2 ml)

**Table 2**

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (ml)</td>
<td>0.9</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Patient's serum (ml)</td>
<td>0.1</td>
<td>0.5 ml serial dilutions</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution</td>
<td>1/10</td>
<td>1/20</td>
<td>1/40</td>
<td>1/80</td>
<td>1/160</td>
<td>1/320</td>
<td>1/640</td>
<td></td>
</tr>
</tbody>
</table>

**5. WARNINGS AND PRECAUTIONS**

For in vitro diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet and the product labelling for information on potentially hazardous components.

**HEALTH AND SAFETY INFORMATION**

1. Handle all bacteria according to appropriate local and statutory guidelines.

2. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for at least 15 minutes at 121°C.

3. Spillage of potentially infectious material should be removed immediately using absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant or 70% alcohol.

4. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

5. These reagents contain phenol. Although the concentration is low, it is known to be toxic by ingestion and skin contact. Avoid ingestion of the reagents. If any come in contact with skin or eyes wash the area extensively by immediately rinsing with plenty of water.

6. In accordance with the principles of Good Laboratory Practice, it is strongly recommended that samples and reagents should be treated as potentially infectious and handled with all necessary precautions.

**ANALYTICAL PRECAUTIONS**

1. Do not use antisera beyond the stated expiry date. Microbiological contamination of the antisera must be avoided as this may cause erroneous results and reduce product life.

2. Do not modify the test procedure, incubation time, or temperatures.

3. After use, return sera to recommended storage temperature (2 to 8°C).

4. The polyvalent antisera are intended for use in slide agglutination tests.

**6. SPECIMEN COLLECTION, TRANSPORT AND STORAGE**

For details of specimen collection and preparation a standard text book should be consulted. The use of fresh cultures on non-selective media is recommended (e.g., nutrient agar).

**7. PROCEDURE**

**MATERIALS PROVIDED**

See Kit Contents.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. 0.85% saline.

2. Glass slides.

3. Microbiological loop and Bunsen burner.

4. Light source over dark background.

5. Test tubes and rack.

6. Waterbath (adjustable) with thermometer.

7. Timer.

8. Pipettes.

9. 0.5% formal saline.

**TEST PROCEDURE**

**Slide Agglutination Test**

**Step 1.** Put two separate drops (40 µl each) of saline on a glass slide. Emulsify portions of the culture under test with a loop in each drop of saline to give a smooth, fairly dense suspension.

**Step 2.** To one suspension add one drop (40 µl) of saline as a control and mix. To the other suspension add one drop (40 µl) of undiluted antisera and mix.

**Step 3.** Rock the slide for one minute and observe for agglutination, which can be more easily seen by viewing against a dark background using indirect lighting. Discard the used slide for safe disinfection.

**Tube Agglutination Test**

**Step 1.** The antigen may be prepared by suspending growth from an agar slant culture in saline or 0.5% formal saline to give a fairly light suspension (about 7.5 x 10⁷ organisms per ml). This should be heated for one hour at 100°C to obtain an O antigen suspension; alternatively a heated four to six hour broth culture of the organisms may be used.

**Step 2.** Make serial dilutions of antisera in 0.5 ml volumes in saline from 1 in 10 to 1 in 640, as shown in Table 2. Discard 0.5 ml from tube 7. Round-bottomed glass tubes approximately 9 mm x 85 mm are suitable.
8. RESULTS

Slide Agglutination
Agglutination should be strong and clearly visible within one minute. There should be no visible agglutination in the control suspension; if agglutination is seen in the control, the suspension is not suitable for testing by this method.

Tube Agglutination
In a positive reaction, there should be obvious granular agglutination. There may be some clearing of the fluid, and a sediment that rises as a granular mass and then sinks again to the bottom of the tube when the tube is flicked with the finger. In a negative reaction and the saline control, the appearance of the suspension should be unchanged and any sediment should resuspend on flicking. Agglutination in the control tube indicates a rough suspension, which is unsuitable for testing.

QUALITY CONTROL
Each laboratory should follow their state and local requirements for quality control testing.

It is recommended to test the product, throughout its use, with known positive and negative cultures.

Homologous cultures should be used for positive control organisms. For a negative control culture, use *Hafnia alvei*. Cultures may be obtained from recognised culture collections such as the NCTC or ATCC.

INTERPRETATION OF RESULTS

Slide Agglutination
Non-specific agglutination, differing in appearance from the specific agglutination, may occur with the slide technique particularly when carried out on bacteria taken from selective media. This agglutination is usually fine and is slow to appear.

Tube Agglutination
The titre of the serum is the last dilution showing positive agglutination. A titre at or near that stated on the bottle label should indicate homology.

Agglutination titres of 1 in 20 are not significant; titres at or near that stated on the bottle label indicate that the antigen is of the same O group as the antiserum.

9. LIMITATIONS OF THE PROCEDURE
The sera are specific for other commonly occurring serotypes of *E. coli*. However, as antigenic components are widely shared throughout the Enterobacteriaceae it is important to confirm any isolate biochemically as well as serologically.

10. EXPECTED RESULTS
Visible agglutination in the presence of homologous cultures.

11. SPECIFIC PERFORMANCE CHARACTERISTICS
See Interpretation of Results

12. BIBLIOGRAPHY