Escherichia coli Antisera

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Product codes BTA521 – BTA548 2ml

Typing antisera as an aid to the identification of Escherichia coli.

Intended Use
Escherichia coli agglutinating sera are intended for use in agglutination tests for the presumptive identification of *E. coli* serotypes. Since antigenic components are shared through many *Enterobacteriaceae* it is important to confirm by biochemical tests that an organism is of the species *E. coli* when attempting serological identification.

Summary and Explanation
Differentiation within the species is made on serological grounds and strains recognised as causing disease fall into a restricted number of serotypes. They are distinguished on the basis of 2 classes of antigens O (Somatic) and K (Surface).
The K antigens possessed by most enteropathogenic strains of *E. coli* belong to the B sub group. They are found on the sheaths or capsules and are inactivated by heat treatment at 100ºC for one hour. This treatment leaves the heat stable O antigens intact. Microgen sera contain both O and K antigens. They have been absorbed to remove cross reactions of other commonly occurring *E. coli* serotypes.

Principle
Liquid stable antisera for the determination of O antigens for the serological identification of pathogenic *Escherichia coli.* 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.

Formulation
Antisera are prepared from rabbits hyper-immunised with standard strains of killed organisms possessing known serotypes or group specific antigens and contain <0.1% sodium azide as preservative.

Warnings and precautions
Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel**.** Sterilise all biohazard waste before disposal. Sodium azide preservative may be toxic if ingested and may react with lead and copper plumbing to form highly explosive salts. Always dispose of by flushing to drain with plenty of water. Refer to Product Safety Data sheet.

Contents: See pack label

Stability and storage
Store unopened at 2-8°C until the expiry date shown on the pack label. Once opened, product should be stored at 2-8°C and may be used until the expiry date given on the label. On storage, some antisera may become slightly turbid. This does not necessarily indicate deterioration and the product may be clarified by centrifugation or filtration before use. If the product exhibits gross turbidity indicating contamination it should be discarded.
Do not freeze reagents
Materials required but not provided:
Standard microbiological supplies and equipment such as loops, applicator sticks, clean glass microscope slides or glass tubes, swabs, culture media, incinerator and incubator, etc., as well as reagents and additives such as sterile 0.85% saline solution.

Procedure

Slide agglutination of heat-treated organisms

1. Prepare a dense suspension of organism to be tested by taking 3 to 5 1-2mm colonies of organisms from a fresh culture on Nutrient Agar or similar and placing in 3ml of 0.85% saline. The suspension should be heated to 100°C for 60 minutes or autoclaved at 121°C for 15 minutes and centrifuged at 900g for 20 minutes. The supernatant should then be removed and 0.5ml of 0.85% saline added to re-suspend the precipitate. Mix the suspension until homogeneous and use this as the antigenic suspension for O-antigen grouping.
2. Place two loopful or drops (5-10µl) of antigenic suspension onto a carefully cleaned microscope slide. The slide may be partitioned using a chinagraph pencil.
3. Place a drop of polyvalent antiserum onto one of the drops of emulsified isolate and on to the other a drop of saline as a control. Note: Do not allow the organism to contaminate the antiserum dropper bottle.
4. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing it under indirect light against a dark background.
5. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination), should be regarded as a positive result. Weak agglutination should be recorded as negative.

If *E. coli* O157 is suspected the following procedure may be used

1. Take a slide as described in 2. above
2. Place one drop of typing serum onto the test area and one drop of saline onto the control area.
3. Using a loop, place a drop of culture onto both the serum and saline.
4. Mix the reagents by tilting the slide back and forth for 60 seconds while viewing it under indirect light against a dark background.
5. Distinct clumping or agglutination within this period, without clumping in the saline control (auto-agglutination), should be regarded as a positive result. Weak agglutination should be recorded as negative.
6. If positive further test with E. coli H7 antiserum as described below.

Interpretation of results
Isolates producing a distinct positive reaction with a polyvalent antiserum are assumed to be an *E. coli* bearing one or more of the O antigenic factors represented by that antiserum.
Further testing of the isolate should be conducted as described in steps 1-3, with monovalent antisera.

Limitations of use
Only cultures of organisms identified as *E. coli* by morphological and biochemical features should be serotyped with this product.
Selective Isolation media should not be used for culturing specimens for O agglutination testing as antigen production may be insufficient or auto agglutination may occur.
Only use heat-treated organisms in the test. This is done to allow identification of the O antigen type as distinct from the heat labile K antigen.
If O157 is suspected, the colonies can be tested directly as the typing serum does not contain K activity.
Polyvalent and monovalent antisera are intended for use in rapid slide agglutination tests only with the exception of H7.
The serotype of an *E. coli* strain is expressed as a combination of O group and H type antigens. H7 antigen determination may require the isolate to be passaged through a motility medium to stimulate flagella production. A suitable procedure is included in the insert for guidance.
O group antigens are not definitively identified by slide agglutination. Definitive identification requires comparison of agglutinin titre against a reference strain by quantitative agglutination.
If more than one monovalent O group antiserum is positive the strain should be confirmed by qualitative agglutination testing.

Quality control
It is recommended that quality control should be performed with at least one positive organism to demonstrate a positive reaction and at least one negative organism to demonstrate a negative reaction. Do not use the product if the reactions with the control organisms are incorrect. Check for signs of deterioration. Do not use reagents if they are contaminated or cloudy.

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Procedure for H Antigen (Tube test)

1. Transfer a loopful of growth from the medium to 2ml of TSB. Suitable test material may be obtained from the moist bottom of a TSI slant where the presence of motile bacteria is most likely. Incubate at 37°C for 6-8 hours.
2. After incubation add 2ml of 1% Formol Saline. Allow to stand at room temperature for 20 minutes. Transfer 0.5ml to each of two 12 x 75mm tubes.
3. To the first tube add 50µl of E. coli H7 antiserum and nothing to the second tube as a negative control. Allow to stand in water bath at 48 – 50°C for 1 hour.
4. Observe each tube for agglutination indicating a positive result. If agglutination/flocculation occurs in the control tube, the isolate is rough and cannot be interpreted by serological means.

E. coli Typing Antisera are listed below.

Cat. No.	Description	Volume
BTA 521	E. coli Polyvalent I	2ml
BTA 522	E. coli O 25 : K 11	2ml
BTA 523	E. coli O 26 : K 60	2ml
BTA 524	E. coli O 44 : K 74	2ml
BTA 525	E. coli O 55 : K 59	2ml
BTA 526	E. coli O 78 : K 80	2 ml.
BTA 527	E. coli O 111 : K 58	2 ml.
BTA 528	E. coli O 114 : K 90	2 ml.
BTA 529	E. coli O 119 : K 69	2 ml.
BTA 530	E. coli Polyvalent II	2 ml.
BTA 531	E. coli O 86 : K 61	2 ml.
BTA 532	E. coli O 124 : K 72	2 ml.
BTA 533	E. coli O 125 : K 70	2 ml.
BTA 534	E. coli O 126 : K 71	2 ml.
BTA 535	E. coli O 127 : K 63	2 ml.
BTA 536	E. coli O 128 : K 67	2 ml.
BTA 537	E. coli Polyvalent III	2 ml.
BTA 538	E. coli O 18a O 18c : K 77	2ml.
BTA 539	E. coli O 20a O 20b : K 84	2ml.
BTA 540	E. coli O 28 : K 73	2 ml.
BTA 541	E. coli O 112a O 112c : K 66	2ml.
BTA 542	E. coli O6	2ml.
BTA 543	E. coli H 16	2ml.
BTA 544	E. coli O78 : K80	2 ml.
BTA 545	E. coli O157	2 ml.
BTA 546	E. coli H7	2ml.
BTA 547	E. coli O1 : K1	2 ml.
BTA 548	E. coli O2	2ml.

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