DebenDiagnosticsLtd

ESCHERICHIA COLI ANTISERA

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Escherichia coli are a group of gram-negative bacilli belonging to the family *Enterobacteriaceae* and considered as one of the bacteria found in normal human intestinal microbial flora. Their serological types are determined in combination with somatic antigens (O group: O1-O173) and flagella antigen (H type: H1-H56). The *E. coli* that cause intestinal infectious diseases including diarrhoea, acute gastritis or colitis are referred to as pathogenic *E. coli,* which are classified into the following 4 groups according to differences in the mode of pathogenicity: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC). Although the identification of pathogenic *E. coli* requires verification of their pathogenicity, pathogenic *E. coli* often have specific serotypes; therefore, typing of the serogroup and serotype is necessary for screening pathogenic *E. coli.*

E. coli Antisera are liquid products of O- and H- sera containing specific agglutinins for serotyping of *E. coli.* O Group sera are prepared by hyperimmunizing healthy pigs (polyvalent sera) or healthy rabbits (monovalent sera) with reference strains of the organisms and with each serotype inactivated by formalin, heating at 56°C for 30 minutes, removing cross agglutinins by absorption, before antiseptic filtration. For the preparation of H-sera, healthy rabbits are immunized with flagella of *E. coli.* Group O sera are used for O-serotyping tests by slide agglutination, and H sera are for H serotyping tests by tube agglutination.

PRODUCT

1. **Group O sera (Set 1)**
   These are liquid products containing specific somatic (O) antibodies (polyvalent sera: pig, monovalent sera: rabbit) of the organisms and 0.08% w/v sodium azide as a preservative.

   51 vials x 2mL (8 vials of polyvalent sera, 43 vials of monovalent sera)

Polyvalent sera	Monovalent sera						
Polyvalent 1	O1	O26	O86a	O111	O119	O127a	O128
Polyvalent 2	O44	O55	O125	O126	O146	O166	
Polyvalent 3	O18	O114	O142	O151	O157	O158	
Polyvalent 4	O6	O27	O78	O148	O159	O168	
Polyvalent 5	O20	O25	O63	O153	O167		
Polyvalent 6	O8	O15	O115	O169			
Polyvalent 7	O28ac	O112ac	O124	O136	O144		
Polyvalent 8	O29	O143	O152	O164			

2. **H-sera (Set 2)**
   These are liquid products containing flagella (H) antibodies (rabbit) of the organisms and 0.08% w/v sodium azide as a preservative.

   22 vials x 5mL (22 vials of monovalent sera)

H sera							
H2	H4	H5	H6	H7	H9	H10	H11
H12	H16	H18	H19	H20	H21	H27	H28
H34	H40	H41	H42	H45	H51		

3. **Group O sera (Alternative)**
   Polyvalent II (alternative) - content types: 026, 055, 0111, 0119, 0126
   Polyvalent III (alternative) - content types: 086, 0114, 0125, 0127, 0128
   Polyvalent IV (alternative) - content types: 044, 0112, 0124, 0142

INTENDED USE

The *Escherichia coli* Antisera are intended for the qualitative serological identification and screening of prepared *Escherichia coli* serotype bacterial cultures, using slide and tube agglutination to detect the presence of bacterial antigens. *Escherichia coli* Antisera are intended for use by trained laboratory personnel.

PRINCIPLE OF MEASUREMENT

When this reagent is mixed with *E.coli* strain which has antigens corresponding to the reagent, an antigen-antibody reaction occurs to produce agglutination. This reaction is macroscopically observed to determine each serotype.

PROCEDURES

1. **Material required but not provided**
   Small test tubes, physiological saline, pipettes, micropipettes and tips, fluorescent light, microbiological loops.
   1) Determination of group O
      Agar media (nutrient agar medium, heart infusion (HI) agar medium: slant or plate medium), autoclave (121°C) or boiling water bath, centrifuge, glass slide, glass pencil.
   2) Determination of H type
      Semi-liquid medium (0.3% semi-liquid medium (e.g: LIM medium) placed in a test tube with a aerophilic cap, in which a sterilized Craigie's tube is inserted), Liquid medium (brain heart infusion (BHI) liquid medium, HI liquid medium: the volume of medium should be at least 10mL), physiological saline containing 1% vol. formalin, water bath (50°C).
2. **Preparation of reagents**
   The antisera are ready for use.
3. **Specimen**
   Cultures of organisms which are derived from a pure culture and identified as *E. coli* by biochemical tests should be serotyped. If the specimen consists of multiple strains, the serotype may not be correctly identified. For determination test of the H type, motile strains should be used.
4. **Procedures**
   A. Determination of the O group
   Determination of the O-antigen is carried out with heat inactivated bacteria using the slide agglutination method.
   1) Suspend an amount of bacterial growth (3-5 times the amount of a match head) in 3mL physiological saline and heat to 121°C for 15 minutes or 100°C for 1 hour. Centrifuge the heated suspension at 900g for 20 minutes, discard the supernatant, suspend the precipitate with 0.5mL physiological saline and use as an antigenic suspension.
   2) Place a drop each of polyvalent and physiological saline (30µL) as a control onto a cleaned glass slide partitioned into several parts with a glass pencil.
   3) Place an antigenic suspension (5-10µL) onto the serum and physiological saline on the slide glass.
   4) Mix the reagents by tilting the glass slide back and forth for 1 minute and the agglutination pattern is observed. Agglutination is grossly observed with light through the slide including fluorescent light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within 1 minute in the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.
   5) If a specimen tests positive with a polyvalent serum, perform steps 2) - 4) above using each monovalent serum contained in the polyvalent serum.

   B. Determination of H type
   Determination of the H-antigen is carried out using the test tube method with the bacteria cultured in liquid media.
   1) Organisms passed through the semi-liquid media with a Craigie's tube 3 - 5 times may be used for inoculation of the preparatory culture in the liquid medium. Then, a cell suspension should be prepared by culturing in the liquid medium at 37°C overnight and adding an equal amount of physiological saline containing 1% w/v formalin.
   2) Put 3 drops of each H-antisera into separate test tubes using the syringe attached to the containers and then add 0.5mL of the cell suspension to each. Use one tube that does not contain the antisera as a control.
   3) After mixing thoroughly, keep the tubes in a water bath (50°C) for 1 hour and observe with the naked eye as to whether agglutination occurs or not. Take care not to agitate the tubes during observation since the agglutinant tends to break up easily. The name of the antiserum that produced the positive agglutination should be taken as the name of the H-antigen of the tested *E. coli.*

PRECAUTIONS

1. Bacterial culture should be performed using indicated media: unselected media. If selected media are used, antigen production may be insufficient or self-agglutination may occur.
2. Heated organisms are used for determination of O group. If untreated organisms were used, it should be noted that they may give false positive or false negative results.
3. When antigen suspension and serum are mixed as a procedure of slide agglutination, the microbiological loop should be sterilized with a flame for each serum to avoid cross-contamination.

INTERPRETATION OF THE RESULTS

1. **Interpretation test of O group**
   Agglutination is grossly observed under transmitted light. It should be first confirmed that no agglutination is found on the reaction with antigenic suspension and physiological saline. Only strong agglutination observed within 1 minute of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative.

Results for polyvalent sera	Determination and additional tests
Any polyvalent sera shows positive.	Determination test of O group is performed using monovalent sera contained within the polyvalent sera which showed positive.
All polyvalent sera show negative.	The specimen is determined not to be serotypes which are included in *Escherichia coli* Antisera.

Results for monovalent sera	Determination
One monovalent sera shows positive.	The name of the monovalent serum which showed positive is interpreted as the O group of tested strain.
Multiple monovalent sera show positive.	Determination is suspended.
All monovalent sera show negative.	The specimen is determined not to be serotypes which are included in *Escherichia coli* Antisera.

Precautions for Interpretation

1) When agglutination is found on the reaction between antigenic suspension and physiological saline, the test is repeated after a colony is reselected.
2) Most positive strains with multiple polyvalent sera are considered to be another serotype which are included in *Escherichia coli* Antisera. For confirmation, the strains should be tested using monovalent sera contained in the polyvalent sera which showed positive.
3) The serotyping of *E. coli* should not be based on the results of polyvalent sera alone. Some isolated strains produce agglutination with polyvalent sera but not with monovalent sera.
4) O serotypes are not definitely identified by slide agglutination. Identification of O group requires the comparison of agglutinin titre with that of a reference strain by quantitative agglutination.
5) When multiple monovalent sera test positive, the strain should be confirmed by quantitative agglutination using reference strains.

2. **Interpretation test of H type**

Agglutination is observed under sufficient light. It should be first confirmed that no agglutination is found between each antigen suspension and physiological saline. Cotton wool-like agglutination observed after the reaction with H serum should be regarded as positive. If homogeneous suspension is still observed, it should be regarded as negative.

Results of H Sera	Determination
One H sera tests positive	The name of the monovalent serum which tested positive is interpreted as the H type in the specimen
Multiple H Sera test positive	Determination is suspended
All H sera test negative	Determination is suspended

Precautions for determination

1) As an aggregate from the reaction of flagella is very fragile, the test tubes should not be shaken during the observation. If agglutination is indistinct after an hour of reaction, it should be determined after an additional hour's incubation.
2) If multiple H sera test positive, the determination test should be repeated after it was confirmed that the bacterium is derived from a pure culture.
3) If all H sera test negative, the strain may possess H types other than the tested types, or the flagella growth may be insufficient.
4) Flagella conditions have a great effect on H-type determination. Even a motile strain that does not test positive in H-type determination could be identified after repeated enhancement of its motility.

PERFORMANCE

1. **Sensitivity test**
   1) O sera: When one drop of the product reacted on a glass slide with a reference strain of a known serotype, granular agglutination was grossly observed.
   2) H sera: When 3 drops of the product reacted in a small test tube with a reference strain of a known serotype, cotton wool-like agglutination was grossly observed.
2. **Specificity test**

In a test performed in a similar manner to the sensitivity test, the antiserum agglutinates only with the reference strain corresponding to the serotype, while in reactions with non-corresponding reference strains, macroscopic agglutination is not observed.

PRECAUTION FOR USE AND HANDLING

1. **General precautions**
   1) This test is for in vitro diagnostic use only.
   2) This kit should only be used by sufficiently trained lab staff.
   3) Antigenic components of *E. coli* are shared widely throughout the *Enterobacteriaceae.* It is important to confirm that an organism used as a specimen is *E. coli* by biochemical test.
2. **Handling precautions**
   1) All specimens, samples and containers coming into contact with samples should be treated as infectious.
   2) If reagents come into contact with skin, mucous membranes or eyes, wash immediately with plenty of water.
   3) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
   4) The reagent should be allowed to stand at 15-25°C for at least 30 minutes before use.
   5) Used containers should not be used for other purposes.
   6) Sera with different production numbers should not be mixed.
   7) The reagent should be used according to the described procedures.
   8) The reagent should only be used for the intended use.
   9) Special precautions should be taken to ensure that the reagent caps are not exchanged.
3. **Precautions for disposal**
   1) The reagent contains 0.08% w/v sodium azide. Sodium azide may react with lead or copper to form explosive heavy metal azides. The reagent should be disposed with a large amount of water.
   2) All specimens, spills, inoculated product and equipment used in this test should be treated with one of the following methods.
      [1] Soaking in 0.1% w/v hypochlorite for 1 hour or more.
      [2] Autoclaving at 121°C for 20 minutes or more.

STORAGE AND SHELF LIFE

Storage: 2-10°C
Shelf life: Up to the expiry date on the label.

PACKAGE

E. coli Antisera: Each type in a 2 mL (group 0 serum), 5mL (H serum) vial with a pipette

- Set 1: O sera 51 vials (8 vials of polyvalent sera, 43 vials of monovalent sera), 1 package
  * Each serum is also separately available.
- Set 2: H sera 22 vials (22 vials of monovalent sera), 1 package
  * Each serum is separately available.
- Polyvalent II (alternative)
- Polyvalent III (alternative)
- Polyvalent IV (alternative)

REFERENCES

1) Supervised by the Ministry of Health, Labour and Welfare: Intestinal pathogenic *E. coli,* Microbiological test manual. Bacterial and fungi tests, Third edition, Japan Public Health Association, D-30 (1987).
2) T. Tsukamoto: *Escherichia coli,* Rinsho-to-biseibutu, 15, 69 (1989).
3) R. Sakazaki: Serotyping of diarrheagenic *E. coli,* Media Circle, 34, 117 (1992).

	Manufacturer
EC REP	Authorised representative in the European Community/European Union
	Consult instruction for use
IVD	For in vitro diagnostic use only
REF	Catalogue number
LOT	Batch code
	Storage temperature limitation
	Use by
LATEX	Contains or presence of natural rubber latex

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