SSI DIAGNOSTICA

Instructions for use

E. COLI ANTISERA

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For *in vitro* diagnostic use

Intended use

Escherichia coli (*E. coli*) antisera are used as an *in vitro* diagnostic aid for qualitative manual complete or partial bacterial serotyping by slide agglutination, for overnight agglutination in round bottom microtiter plates and/or agglutination in Widal tubes.
It is important to use pure culture isolates for determination of bacterial antigens.

Description

E. coli O, H, K9 and F antisera are for full serotyping, whereas OK O antisera are for screening.
O antisera are intended for serotyping with boiled culture. H antisera are intended for serotyping with formalin killed culture, and K9, F and OK O are for sliding of live cultures from a non-selective agar plate.

Antisera type	Vial volume	Number of tests
OK O pool and OK O single	3 mL	150
O pool and O single	3 mL	35
O single	1 mL	12
O single, high titer	1 mL	48
H pool	3 ml	30
H single	5 mL	50
K9	1 mL	50
Fimbrial antisera	5 mL	250

Table 1. Products included in this instruction for use.

All *E. coli* antisera are absorbed free from cross-reactions. For OK O pool antisera, the pools are only absorbed for serotypes contained within the other OK O pools.

The antisera are polyclonal, prepared in rabbits using reference strains according to the methods recommended by the World Health Organization and absorbed to eliminate cross-reacting antibodies.
E. coli antisera are for use by laboratory professionals and/or healthcare professional only.

***E. coli* OK O antisera** OK O antisera are for screening of live cultures from a non-selective agar plate. The pools have been absorbed free from cross-reactions against each other (pool 1 will not cross-react with serotypes from pool 2 and 3 etc.), while the single antisera have been absorbed free from cross-reactions towards the serotypes included in its corresponding pool. The OK O result is a screening result. It is important to have the O group confirmed by agglutination with O antisera using a boiled culture. The available OK O antisera are used to detect the most frequent and pathogenic *E. coli* for humans.[1]

***E. coli* O antisera** O antisera are for serotyping of boiled cultures. Boiling of an *E. coli* culture removes heat stable antigens on the cell surface. These antigens can cover the O antigens and are possible cross-reactions against other antigens than O antigens. All O single and pool antisera are absorbed free from cross-reactions against other O antigens. Some strains (e.g. O8, O9, O20 and O101) have heat-stable acidic capsules (K antigens) resembling lipopolysaccharide O antigens and these may sometimes cover the O antigen. Such strains can only be serotyped after autoclaving a broth culture at 121 °C for 2 hours. The ready to use O antisera should not be diluted, while the O high titer antisera must be diluted in physiological saline, pH 7.4 as described on the label. [1]

***E. coli* H antisera** H antisera are for H serotyping of formalin killed culture. The formalin fixates the H antigen, making an agglutination easier to see. Use a fume cupboard when working with formalin.[1]

***E. coli* K9 antisera** K9 antiserum is for screening of live cultures.

***E. coli* fimbrial antisera** F antisera are for screening of live cultures from a non-selective agar plate (for detection of F4 antigen) or from a Minca IS agar plate (for detection of F5 antigen). False positive and false negative results might occur if bacteria from another selective medium is used. The F4 and F5 antisera are the most common fimbrial adhesins, which help ETEC to bind to the intestinal mucosa.

Principle

Antigen-antibody complexes are formed (agglutination) when a bacterial culture is mixed with a specific antiserum directed against bacterial surface components. The complexes are usually visible to the naked eye which allows for easy determination of O, H, K and F antigens by agglutination[1].

Precautions

- Before using SSI Diagnostica *E. coli* antisera, confirm that the strain is an *E. coli*, e.g. by using a biochemical method.
- Rough cultures/strains will self-agglutinate and cause false positive reactions.
- Excessive amount of culture compared to antisera might cause false positive reactions.
- For the antisera for slide agglutination, please make sure that the result is read within 10 seconds.
- Turbidity due to lipoprotein precipitation can occur after prolonged storage. If you experience precipitation and/or contamination, it can be removed by centrifugation (10,000 g) followed by sterile filtration (0.22 µM).
- The antisera have only been validated for serotyping by the below described methods.
- Antisera that have accidentally been frozen should not be used.
- The strain to be tested must be grown on a non-selective agar plate. Be sure that the strain is a pure culture.
- Do not use the antisera after the expiry date.
- Inspect the vial before use to ensure it is intact. Any damaged vials should be discarded.

Materials provided

SSI Diagnostica *E. coli* antisera are supplied in dropper bottles containing 1, 3 or 5 mL of ready-to-use antisera. *E. coli* high titer antisera are supplied in dropper bottles containing 1 mL, which must be diluted 1:4. (see table 1).

Materials required but not provided

- Non-selective agar medium (e.g. beef extract agar)
- Physiological saline pH 7.4
- Inoculating loop or toothpick
- Pipettes
- Incubator (35-37 °C)

Additional for OK O and K9 antisera

- Glass slide

Additional for O antisera

- Infusion broth
- Round bottom microtiter plate
- Foil bag
- Incubator (50-52 °C)
- Water bath (100 °C)

Additional for H antisera

- Infusion broth (e.g. *E. coli* broth)
- Semisolid agar
- Transfer pipettes
- Glass tubes (Widal tubes)
- Water bath or incubator (50-52 °C)

Additional for F antisera

- Minca IS agar plates (IS = Iso Vitalex)
- Glass slides

Storage and stability

Expiry date is printed on the label.
E. coli antisera should be stored at 2-8 °C in a dark place. Do not freeze. Stored under these conditions the antisera may be used up to the date of expiry shown on the product label.

The in-use stability is not affected by working with the antiserum on the bench throughout the day if it is stored at 2-8 °C when not in-use, for no longer than 4 years from date of production except for H pool antisera, which have 3 years shelf life.

E. coli antisera have been tested after being stored at 37 °C for up to four weeks. The antisera were still fully functional.

Preservative

The *E. coli* antisera contains less than 0.1 % sodium azide (NaN3) as preservative.

Sample collection and storage

For sample storage please follow your local standard procedure.

Quality control

Before use check the vial to ensure there is no damage and/or leak. In case of damage or leak discard the vial.

Saline is used as negative control to confirm that the strain is not self-agglutinating.

Procedure

Slide agglutination with OK O, K9 and F antisera

Preparing test material

1. For OK O and K9 antisera: The *E. coli* is grown overnight at 35-37 °C on a suitable growth medium.
   For F antisera: The *E. coli* is grown over night at 35-37 °C on a suitable agar medium not inhibiting motility and on a Minca IS agar plate (F5 antiserum can only grow on this special medium).

Performing the slide agglutination

1. Apply a small drop of antiserum (approximately 20 µL) on a glass slide.

2. Transfer culture from 3-5 colonies to the drop of antiserum and mix well. The amount of culture should be sufficient to give a distinct milky turbidity. Use an inoculating loop.

3. Tilt the slide for 5-10 seconds.

4. The reaction is read with the naked eye by holding the slide in front of a light source against a black background (indirect illumination).

5. A positive reaction is seen as visible agglutination. A negative reaction is persistence of the homogeneous milky turbidity. A late or weak agglutination (after 10 seconds) should be considered negative (see figure 1).

6. A positive reaction indicates that one of the 3-5 colonies is positive. It is important to identify the positive colony for further analyses to avoid working with mixed cultures.

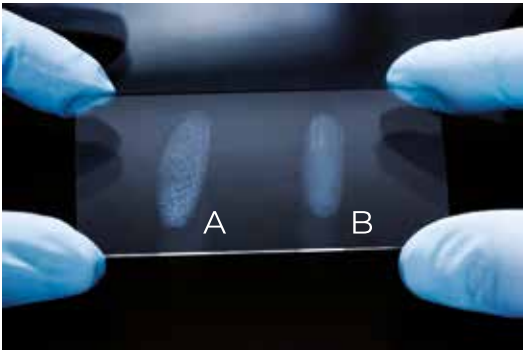

Figure 1. Sample A is a positive reaction and sample B is a negative reaction.

Agglutination with O antisera

Preparing test material

1. It is important to use a broth which does not agglutinate with the antisera, giving false positive results. It is recommended to test the broth before use since different broths agglutinate differently (see figure 2).

2. The positive colony from the OK O screening or 3-5 colonies from a non-selective agar plate are selected for inoculation in infusion broth and incubated overnight at 35-37 °C.

3. Next day the broth culture is boiled (>90 °C) for 1 hour. After boiling, the culture must be left on the table for 1 hour to allow sedimentation. Boiled cultures can be stored at 2-8 °C for 1 week but must be mixed again by turning the broth up-side-down a few times and left for 1 hour on the table before use.

High titer O antiserum must be diluted before use. Recommended working dilution is printed on the label.

Performing the O serotyping

1. Mix 80 µL O antiserum with 80 µL boiled culture in one well of the microtiter plate.

2. Perform a negative control by mixing 80 µL of physiological saline pH 7.4 with 80 µL boiled culture.

3. The lid is placed on the microtiter plate, and the plate is sealed in a foil bag and incubated at 50-52 °C overnight.

4. Next day the reaction is read against artificial light with a black background. The microtiter plate must not be shaken before reading the reaction.

5. A positive reaction is read as a “grey carpet”, covering the bottom of the well. When the reaction is negative the bacterial suspension is a small white spot centered at the bottom in the well (see figure 2).

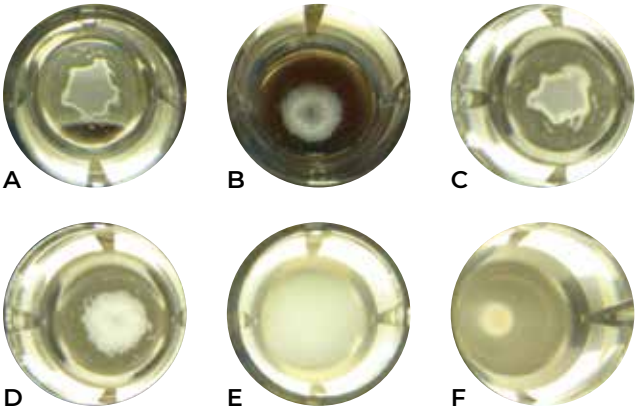

Figure 2. A-E are positive agglutinations of the same antigen and antiserum but with various broths. A: TSB (Tryptone Soya), B: Mueller Hinton, C: Todd Hewitt, D: Brain heart infusion, E: Beef broth. F is a negative control where physiological saline is mixed with a boiled beef broth culture.

Agglutination with H antisera

Preparing test material

1. Day 1: 3-5 colonies from a non-selective agar plate are selected for inoculation in a semisolid agar (approximately 2-3 cm deep) and incubated overnight at 35-37 °C.

2. Day 2: Use a transfer pipette to pass 3-5 drops of the culture (which has grown furthest down towards the bottom of the tube) on to another semisolid agar and incubate overnight at 35-37 °C.

3. Day 3: If the culture has reached the bottom of the semisolid agar, transfer 3-5 drops from the bottom of the semisolid agar into a nutrient beef broth using a transfer pipette, and incubate for 6 hours at 35-37 °C*. If the culture has not reached the bottom, repeat step 2. Generally, two passages in semisolid agar are sufficient. Occasionally, three or more passages in semisolid agar may be helpful. Figure 3 illustrates the motility of *E. coli* in semisolid agar.

4. Add formalin to a final concentration of 0.5%** to fixate the H antigens.

5. Leave for 30 minutes before use.

*Motility may be further improved if incubated at 30-37 °C in a rotating drum set at an angle of 45° for 5 to 6 hours.
**Volume of 37% formalin which should be added to a broth to obtain a final concentration of 0.5% formalin. Remember to work in a fume cupboard.

Broth to formalin ratio				
mL broth	5	10	15	20
mL 37% formalin	0.07	0.14	0.20	0.27

Table 2. Broth to formalin ratio to reach a final concentration of 0.5% formalin

Performing the H serotyping

1. Mix 3 drops (approximately 180 µL) H antiserum with 180 µL formalin fixed culture in a glass tube.

2. For a negative control mix 3 drops (approximately 180 µL) of physiological saline pH 7.4 with 180 µL of formalin fixed culture in a glass tube.

3. The glass tubes are incubated in a humid atmosphere (water bath or incubator) at 50-52 °C for 1½ to 2 hours (maximum). Do not shake or rock the tubes when removing them.

4. After a very gentle tilt of the tubes the reactions are read immediately against artificial light with a black background (indirect illumination). The reactions will disappear within 2-3 seconds after the tubes have been tilted.

5. The typical H agglutinates are loose and fluffy. The shape and structure can, however, vary depending on the influence of the other antigens present.

6. A negative reaction is seen as a milky turbidity with no agglutination.

Please note!
Some H serotypes (e.g. H32) can be difficult to serotype using the traditional agglutination assays described above. WHO has approved and recommends a motility test for serotyping using SSI Diagnostica antisera, when traditional agglutination causes difficulties.
Presumed non-motile cultures are incubated at room temperature (18 °C) overnight and subcultured in semisolid agar to improve motility. A strain can only be defined as H-rough if a motility test is negative.

When performing a motility test (using a microscope), antibody and bacteria are mixed on a glass slide. The mixture is covered by a cover slip. A positive agglutination appears as clots in a solution of free-swimming bacteria. A negative test result (no agglutination) appears as free-swimming bacteria without clots. It is important to use an equal amount of antigens and antibodies.

Figure 3. A) Non-motile *E. coli* (fluid is clear). B) A motile *E. coli* which has grown almost halfway down (fluid is cloudy where it has grown). It needs to be subcultured to reach full motility, before serotyping the H antigen. C) The fully motile *E. coli* which has grown all the way down (fluid is cloudy).

Figure 4. Positive H-agglutination

Interpretation of results

Slide agglutination with OK O, K9 and F antisera

A positive reaction is seen as a visible agglutination, whereas a negative reaction is persistence of the homogeneous milky turbidity (see figure 1). Do not interpret the results after 10 seconds as any reaction seen after 10 seconds cannot be considered a true positive result.

Agglutination with O antisera

A positive reaction is read as a “grey carpet”, covering the bottom of the well whereas a negative reaction is read as a small white spot centered at the bottom in the well (see figure 2).

Agglutination with H antisera

The typical H agglutinates are loose and fluffy. The shape and structure can, however, vary depending on the influence of the other antigens present. A negative reaction is seen as a milky turbidity with no agglutination (see figures 3 and 4).

Disposal

Follow your local procedures and/or national guidelines for disposal of biological materials.

Limitations

- The culture must be confirmed *E. coli* before serotyping using antisera from SSI Diagnostica.
- Antisera can only be used as described in this IFU.

Performance

Sensitivity, specificity, and repeatability

***E. coli* antisera overall results**		
	Percent (number positive/actual positive)	95% confident interval
Sensitivity	99% (234/236)	97-100
Specificity	98% (334/340)	96-99
Repeatability	99% (862/870)	98-100

Table 3. Sensitivity, specificity, and repeatability

Reproducibility

The reproducibility within the different groups of antisera and all antisera combined is 97.47% (95.25% - 98.66%). Therefore, all produced antisera have a high level of reproducibility throughout time and lots.

Incident reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Quality certificate

SSI Diagnostica's development, production and sales of *in vitro* diagnostics are quality assured and certified in accordance with ISO 13485. Certificate of analysis can be downloaded from our website: ssidiagnostica.com

IVD

REF

For the list of products and composition, see our website:
- https://ssidiagnostica.com/nordic/solutions/antisera/e-coli-antisera/

References

1. Ørskov *et al*., Bacteriol Rev. 1977 september; 41(3):667-710.

Information and ordering

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