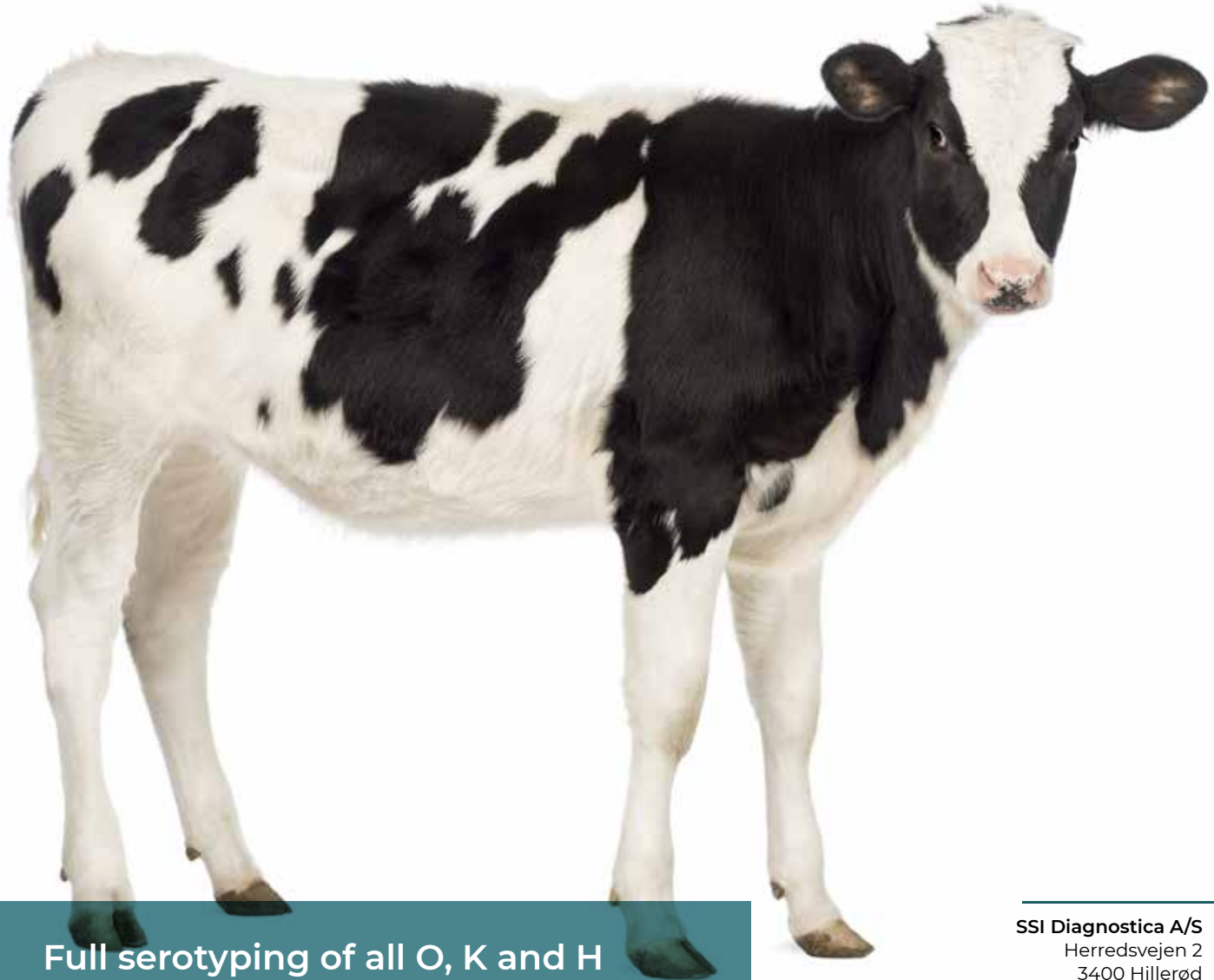
SSI DIAGNOSTICA

E. COLI ANTISERA



Full serotyping of all O, K and H antigens plus the most frequent bacteriophages and F antigens.

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Improving
Microbiology

CONTENT

INTRODUCTION

Background

Escherichia coli (*E. coli*) is among the most frequent causes of food related diarrhoea. Most *E. coli* strains are under normal circumstances harmless and part of the indigenous intestinal flora in warm-blooded organisms, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. Big outbreaks in the industrialized world have proved the need for serotyping of *E. coli* as a base for proper diagnosis, identification, surveillance, and food control.

All humans and animals carry *E. coli* bacteria in their intestines. However, there are several types of *E. coli* strains that may cause gastrointestinal illness. In humans these strains can be divided into seven groups or pathotypes:

- Enteropathogenic *E. coli* (EPEC)
- Attaching and effacing *E. coli* (A/EEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterohaemorrhagic *E. coli* (EHEC)
- Enteroaggregative *E. coli* (EAggEC)
- Extraintestinal pathogenic *E. coli* (ExPEC)

E. coli is a Gram-negative, facultative anaerobic, and non-sporulating rod-shaped bacteria. The genus consists of more than 100.000 different serotypes described by Fritz Kauffmann, Ida Ørskov, and Fritz Ørskov in 1964-1992 during their work at Statens Serum Institut (SSI), WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*(1). Determination of O (lipopolysaccharide), H (flagella, protein), K (polysaccharide, capsule) and F (fimbria, protein) antigens by using specific antisera, enables identification of the antigen combination and thus differentiation of the many serotypes (see figure 1). The serotype is not equivalent with certain virulence genes, but it gives some indication, and it is important especially in research of outbreaks.

For more than 80 years, SSI has been engaged in the development of the *E. coli* typing system and in the production of *E. coli* antisera.

As the only company in the world, SSI Diagnostica offers the full product range of *E. coli* antisera for determination of all serotypes described so far.

SSI Diagnostica worked closely with the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* at SSI, to ensure development of up-to-date products and guidance of clinical and epidemiological relevance.

Description

This brochure focuses on the SSI Diagnostica *E. coli* antisera, intended for serological confirmation and serotyping of identified *E. coli* strains. Our product range includes almost 400 different *E. coli* antisera, which are divided into O, H, K, F and OK O antisera and the corresponding pool systems.

All our antisera are polyclonal and are produced by immunisation of rabbits with well-defined reference strains. For antisera, wherever practicable, the reaction is made specific by absorption. All products are tested against a large number of strains.

Our antisera are supplied as "ready to use" (1, 3, and/or 5 mL) and for O antisera as "high titer" (1 mL). All antisera are supplied in user-friendly vials. The number of tests for each product is stated in the product catalogue and on our website ssidiagnostica.com.

Figure 1. Illustration of *E. coli* bacteria

Assortment

SSI Diagnostica offers a full range of *E. coli* antisera which are listed in table 1. Besides the antisera, we also provide a wide range of related *E. coli* products. These include:

- K1, K5 and K13 bacteriophages
- K9 for slide agglutination
- DEC PCR
- *E. coli* strains for O, K, H and F antigens in addition to various toxins and virulence factors

All products are produced at our production premises in Hillerød in Denmark and certified in accordance with ISO 13485 except for our *E. coli* K antisera, which are for research use only.

Advantages of SSI Diagnostica's *E. coli* antisera:

- Full range of antisera for *E. coli* serotyping.
- Reaction time for OK O antisera is only 10 seconds.
- SSI Diagnostica antisera are absorbed and thus very specific.
- SSI Diagnostica provides technical expertise.

Product group	Type of culture	Method	Use
O pool antisera (AA-XX)	Boiled	Agglutination in microtiter plate	Full serotyping
O single antisera (O1-O187)	Boiled	Agglutination in microtiter plate	Full serotyping
OK O pool antisera (1-3)	Live	Slide agglutination	Screening
OK O pool "Big Six"	Live	Slide agglutination	Screening
OK O single antisera (various)	Live	Slide agglutination	Screening
H pool antisera (A-F)	Formalin-killed	Agglutination in test tube	Full serotyping
H single antisera (H1-H56)	Formalin-killed	Agglutination in test tube	Full serotyping
K single antisera (K2-K103)	Bacteria extract	Counter current electrophoresis	Full serotyping
F single antisera (F4 and F5)	Live	Slide agglutination	Full serotyping

Table 1. SSI Diagnostica assortment of *E. coli* antisera

SCREENING

Several types of *E. coli* strains may cause gastrointestinal illness among humans and animals. At SSI Diagnostica antisera is produced for screening of both human and animal isolates.

Screening is a fast and cost efficient way of serotyping compared to a full serotyping. It can be used where the origin of a strain is known or where it is possible to exclude or screen for highly patogenic *E. coli* strains.

Screening in humans

For clinical use we recommend the OK O pools and single antisera.

With the OK O antisera you get a result within 10 seconds, but all OK O antisera need to be confirmed by O antisera for use with boiled culture (see table 2.).

OK O pool	
1	EPEC/VTEC/STEC
2	EPEC
3	EPEC
Big Six	EPEC/VTEC/STEC

Table 2. OK O pool antisera for human screening

Screening in animals

In addition to screening for *E. coli* O157:H7, regulations in the United States require the meat industry to monitor for six Shiga toxin-producing *E. coli* (VTEC) serogroups, the so-called "Big Six" VTEC (O26, O45, O103, O111, O121, and O145). SSI Diagnostica offers an OK O pool containing these six serogroups for quick slide agglutination.

For the composition of the O pools see SSI Diagnostica's product catalogue or our website ssidiagnostica.com.

Below, figure 2 shows the screening method which SSI Diagnostica recommends, when an *E. coli* strain has been confirmed.

Screening method:

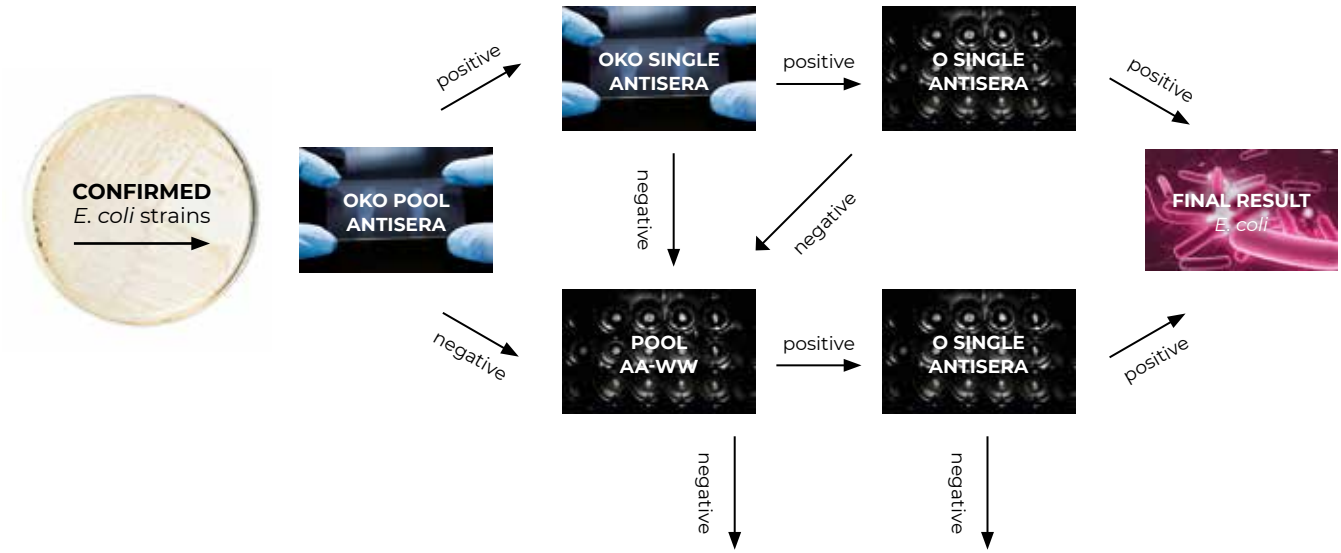

Figure 2. Flow chart illustrating serotype screening of *E. coli* strains using antisera from SSI Diagnostica.

- Not an *E. coli*
- It might be a rough *E. coli*
- The capsule might be heatstable and can only be removed by autoclavation
- The broth used is not suitable for agglutination assays

FULL SEROTYPING

A full serotyping is determined as a serotyping of O, H and sometimes K antigens. A complete serotyping is performed when screening has not given an indication of a serotype, or if the origin of the sample is multiple. Additionally, it is performed during outbreaks or in severe cases of illness.

O serotyping

SSI Diagnostica produces *E. coli* O antisera for boiled cultures. The O antisera panel consists of O pool AA-XX and the relevant O single antisera. This pool system for full serotyping is based on antigen similarity and often used by reference laboratories. Serotypes with similar antigens are grouped in the same pools to limit false positive results. The pool system result should be specified by agglutination with O single antisera.

Recommendations for O full serotyping

A rough *E. coli* strain might not agglutinate with any O antisera. A rough strain is very mucoid with the characteristic "pie cut". If you suspect to have a rough strain, take a small amount of the colony from a non-selective agar plate and suspend it in distilled water instead of broth. The colony density should be similar to an overnight broth. Boil the suspension for at least an hour, and use it for O serotyping, the same way that you would have used the boiled broth. If this serotyping is unsuccessful try to cultivate the strain on a new non-selective agar plate which has been flushed with ethanol and dried. Repeat the procedure for the suspension and boiling as described above. This method will often make it possible to serotype the O antigen on a rough strain.

Another possibility is to cultivate a rough strain in semi solid media and strike it on to an agar plate.

Some strains have a heat stable capsule. These strains are very often O serotype O8, O9, O20 or O101 and must be autoclaved instead of boiled.

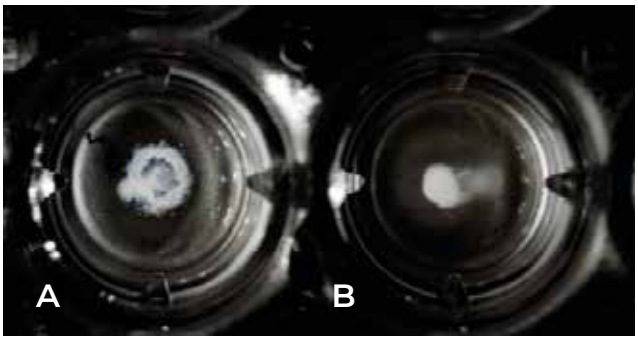

Figure 3. A positive (A) and a negative (B) agglutination test for *E. coli* O antigens using O antisera for boiled culture

When serotyping by agglutination, it is very important to perform a negative control in parallel with your sample. As shown in figure 3, a very strong positive sample can appear as a dot, but when comparing to the negative control the difference is obvious.

At SSI Diagnostica, serotyping is performed using beef broth. Different media have different impact on the agglutination.

It is very important to find media, which do not interact with the antigens or the antibodies. False negative or false positive reactions may occur due to the media (see figure 4 below). Note that a positive agglutination often appears in a clear fluid, whereas a negative can appear as a dot in either a cloudy or a clear fluid.

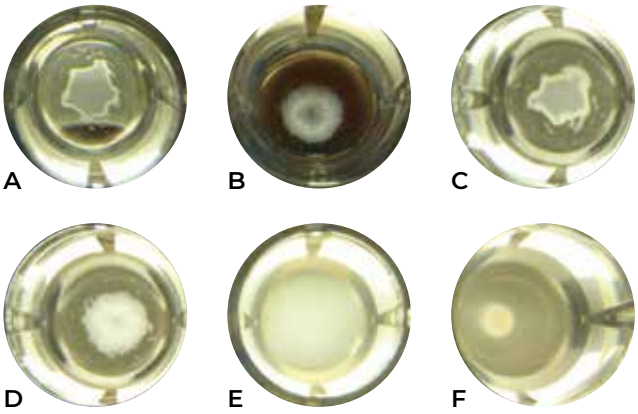

Figure 4. Positive O agglutination using the same antisera and the same *E. coli* strain, but cultivated in different media
A: TSB (Tryptone Soy), B: Mueller Hinton, C: Todd Hewitt,
D: Brain Heart Infusion, E: Beef Broth, F: Negative

H serotyping

As for the full serotyping of O antigen, the H serotyping is performed by agglutination with the pool antisera (H pool A-F) followed by agglutination with the corresponding single antisera.

It might be difficult getting *E. coli* strains to express their flagellar. Sometimes a strain needs to be subcultured in semisolid agar 5-10 times before it begins expressing its flagellar. Each time the culture is subcultured in semisolid agar, it is important that the most motile bacteria (those that have grown furthest down in the tube) are subcultured in a new semisolid agar tube (see figure 5).

If a strain does not express flagellar, it might be an idea to leave the semisolid agar at room temperature for two days.

Bacteria coding for the H56 needs to grow at 30 ˚C before the bacteria expresses the H56 fimbria.

The only way to confirm that a strain is H negative, is to perform a mobility test in a microscope.

An O reaction could theoretically arise during an H test. H antiserum is not absorbed for O antibodies. An O reaction is very rarely seen, since H tests are performed using unboiled cultures, and O antigens are often hidden or partially hidden in a capsule. Additionally, appearance of an O agglutination takes longer than an H agglutination. Each of the two agglutination types can be distincted visually. The *E. coli* positive H is visible as a cloud whereas the O reaction agglutinates as grains. If in doubt, shake the Widal glass containing the H reaction. H reactions are very sensitive to shaking and will immediately vanish. O agglutinations are more stable and will not be destroyed by gentle shaking.

Figure 5. Motility of *E. coli* bacteria

A. A non-motile *E. coli* (fluid is clear).
B. A motile *E. coli* which has grown almost half way 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).

K serotyping

SSI Diagnostica offers all 67 known K single antisera.

Recommendations for K full serotyping

We advise testing for the most prevalent K serotypes first. Based on the O and H serotypes, another possibility is to find the most frequent complete serotypes, and test these combinations to begin with.

K serotyping using current counter electrophoresis can be difficult for some strains. Hence it might be necessary to deviate from standard procedures. If agglutination does not appear or is faint, it can be helpful to dilute the bacteria in a 1:25 solution instead of a 1:50 solution.

If the gel is left overnight in a humid atmosphere, the agglutination will be more pronounced.

A strong agglutination can also be promoted by an extract from TGEX agar plates instead of beef heart agar, or by incubating the plate for two days, and not for one day.

METHODS

Serotyping methods

Serotyping is the classical method for the identification of *E. coli* strains and is still used as the main diagnostic tool.

Antigen-antibody complexes (agglutination) are formed when a bacterial culture is mixed with the corresponding antiserum directed against bacterial surface components. The complexes are usually visible to the naked eye, which allows for easy determination.

A serotype is not indicative of the presence of relevant virulence genes, but it gives an indication of which isolates should be selected for further specific virulence testing.

All *E. coli* strains have O antigens, most strains have H antigens and some have K and/or F antigens. The different serotyping methods are described on the following pages.

Slide agglutination of live cultures using OK O antisera

SSI Diagnostica has developed OK O antisera in order to screen for the most virulent *E. coli* strains belonging to the pathogenic groups: VTEC and/or EPEC. The OK O antisera will be updated whenever new virulent strains become important.

Application

OK O polyclonal antisera are produced by immunisation of rabbits with whole formalin-killed cells. The antisera therefore contains antibodies against O, K, H and other protein antigens found on the surface of the cell. For this reason, OK O antisera are intended only for screening and must always be confirmed with O serotyping. In addition to the OK O single antisera, SSI Diagnostica also offers an OK O pool system.

Procedure

OK O antisera are used for slide agglutination with live cultures, and the reaction can be read after 5-10 seconds.

1. Add a small drop of antisera (approx. 20 µL) on a glass slide and mix it with the *E. coli* culture.

2. Tilt the slide for 5-10 seconds.

3. A positive reaction is seen as a visible agglutination in a clear fluid, whereas a negative reaction is seen as a homogenous milky turbidity (figure 6).

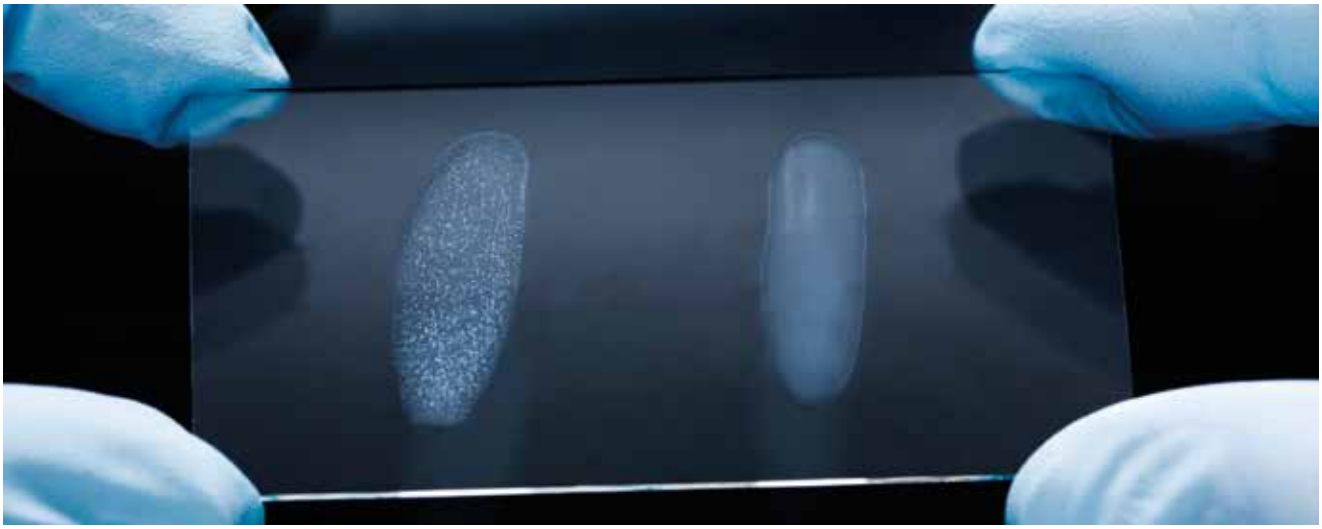

Figure 6. Positive agglutination left, negative agglutination right

Agglutination in microtiter plates for boiled cultures using O antisera

O antigens are thermostable lipopolysaccharides (LPS) found in the outer membrane of *E. coli*. Today 184 different O antigens are known, designated O1 to O187. SSI Diagnostica offers antisera to identify all of them.

Some O antisera cross-react with antigens from other bacteria, e.g. *Shigella* and *Salmonella*, as the O antigens from these species are partly or entirely identical. Before serotyping, it is therefore important that the identification of *E. coli* is confirmed, e.g. with biochemical reactions.

Application

O antisera from SSI Diagnostica consist of polyclonal antibodies produced by immunisation of rabbits with boiled cells. Since the boiling removes all H, K and F antigens, cross-reactions with these are rarely seen.

Some O antisera are sold as monospecific, as the cross-reactions have been removed by absorption. The monospecific single antisera are used for definitive typing in cases where both a non-absorbed and an absorbed antisera gives a positive agglutination.

In addition to the O single antisera, SSI Diagnostica also offers an O pool system (pool AA-WW). Selected single antisera are additionally offered as high titer products for dilution (minimum 1:4).

Procedure

O antisera are used for agglutination of boiled cultures in round bottom microtitre plates. As an alternative Widal tubes can be used. Equal quantities of antiserum and culture are mixed, and the result is read after overnight incubation at 50-52 °C.

1. Add 80 µL *E. coli* culture and 80 µL antisera to a microtiter well (180 µL of each if Widal tubes are used).

2. Incubate the microtiter plate or Widal tube in a humid atmosphere at 50-52 °C overnight.

3. In microtiter plates a positive reaction is seen as a "grey carpet", covering the bottom of the well, often in a clear fluid. When the reaction is negative the bacterial suspension is seen as a small white spot in a clear or a milky turbidity centred in the well (figure 7). Widal tubes must be vigorously shaken before a positive reaction is seen as a granular reaction, and a negative reaction as a persistence of the homogeneous milky turbidity.

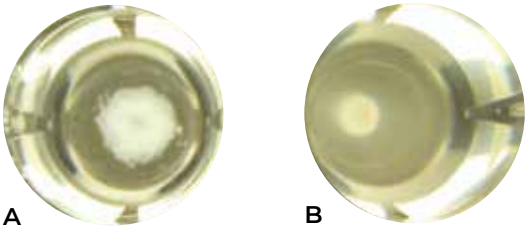

Figure 7. A positive (A) and a negative (B) reaction in microtiter plate

Test tube agglutination with formalinkilled cultures using H antisera

H antigens are proteins found in the outer membrane of *E. coli* and are primarily responsible for the motility of the bacteria.

Even if a particular bacteria has a genomic material to express H antigens, these are generally only expressed if necessary. At the same time, the quantity of H antigens can vary. It is therefore important that only those strains with known motility are H serotyped. 53 different H antigens are known, designated H1 to H56, and SSI Diagnostica offers antisera to identify all of them.

Some H antisera cross-react with antigens from other bacteria, e.g. *Citrobacter*, as the H antigens from these species are partly identical. Before serotyping, it is therefore important that the identification of *E. coli* is confirmed, e.g. with biochemical reactions.

Application

H antisera are polyclonal antibodies produced by immunisation of rabbits with whole formalin-killed cells containing as much flagellum antigen as possible. All H antisera are absorbed and therefore monospecific. Cross-reactions with O antigens can occur, if the reaction is read after the recommended 2 hours.

Only some *E. coli* strains will be actively motile on ordinary agar plates. However, it is usually possible to get well flagellated bacteria by subculturing the organism in a semisolid agar medium.

In addition to the H single antisera, SSI Diagnostica also offers an H pool system.

Procedure

H antisera are used for agglutination of formalinkilled cultures with a final concentration of 0.48% in test tubes. Equal quantities of antiserum and culture are mixed, and the result is read after 1½-2 hours' incubation at 50-52 °C.

1. Add 180 µL *E. coli* culture (formalin-killed culture) and 180 µl antisera to a test tube.

2. Incubate the test tubes in a humid atmosphere at 50-52 °C for 1½-2 hours.

3. Be careful not to shake the test tubes. A positive reaction is seen as a loose and fluffy cloud in a clear fluid (see figure 8). A negative reaction is seen as a homogenous milky turbidity.

Figure 8. Positive H-agglutination

Slide agglutination of live cultures using F antigens

Fimbrial adhesins (F antigens) are proteins found in the outer membrane of *E. coli* and are primarily responsible for adhesion. F antigens help enterotoxigenic *E. coli* (ETEC) to bind to the intestinal mucosa. This way they can severely disrupt the intestinal function and cause diarrhoea in infants and newborn piglets.

Application

F antisera from SSI Diagnostica are polyclonal antisera produced by immunisation of rabbits with formalinkilled cells that express as many fimbriae as possible. F antisera are made specific by absorption. Currently we offer F4 and F5 , which are the most frequent fimbria.

Procedure

F antisera are used for slide agglutination with live cultures, and the reaction can be read after 5-10 seconds. The procedure is similar to slide agglutination using OK O antisera (see page 11).

Current counter electrophoresis using K antisera

K antigens are acidic capsular polysaccharides (CPS), (a thick, mucous-like layer of polysaccharide) that surrounds some pathogenic *E. coli*. The capsules can have various shapes and be more or less heat stable. SSI Diagnostica offers all 67 different K antigens, designated K2 to K103.

Application

K antisera are polyclonal antibodies produced by immunising rabbits with whole formalin killed cells expressing as much capsular polysaccharide as possible.

Procedure

K antisera are used for current counter electrophoresis in an agarose gel. Equal amounts of antisera and bacteria extract are used, and the electrophoresis runs for 50 min at 100V.

1. A bacterial extract is made from a TGEX agar plate.
2. Two rows of 4 mm holes are made in an 1.5% LSA agarose gel.
3. In the upper row approx. 15 µL antisera are applied to each hole.
4. In the lower row approx. 15 µL bacterial extract are applied to each hole.
5. The electrophoresis runs at 100V for 50 min.
6. Positive results are visually read as precipitates between the wells with antiserum and extract. For negative tests no precipitates are seen (see figure 9).

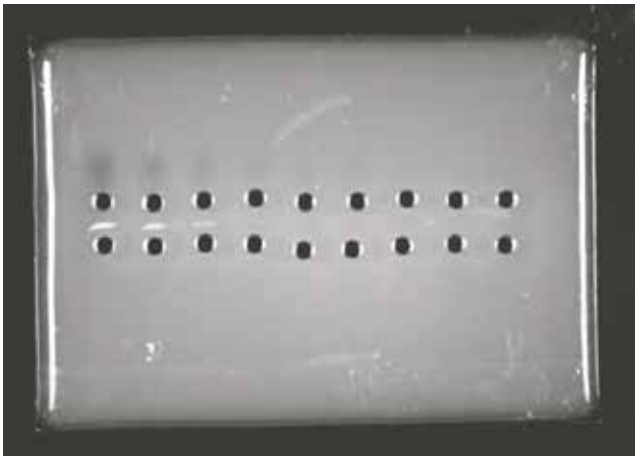

Figure 9. A positive test is marked by a precipitate between the wells (1-4). No precipitate marks a negative test (5-9)

OTHER E. COLI PRODUCTS

E. coli bacteriophages

In addition to our diagnostic antisera, SSI Diagnostica also offers specially produced bacteriophages for phage typing of K1, K5, and K13. Phage typing is easy to perform and can under normal circumstances be carried out within 24 hours.

Application

Bacteriophages are viruses that solely infect and lyses bacteria expressing a specific antigen. Phage typing is used to serotype *E. coli* capsule antigens for which it is not possible to produce antibodies in mammalian, because the capsule antigens resemble the epithelial cells.

Procedure

E. coli cells infected with K1, K5, or K13 bacteriophages are propagated and concentrated, followed by harvesting of the bacteriophages.

1. A streak of phage solution (K1, K5, or K13) is deposited diagonally on an agar plate.

2. Streak the bacterial culture vertically.

3. Incubate the plate overnight at 37 °C.

4. A positive reaction is seen by no or very limited growth after crossing the phage line. A negative reaction is seen by growth in full length of the bacterial streak (see figure 10).

Figure 10. Serotyping of *E. coli* K antigens using bacteriophage suspensions

Strains

SSI Diagnostica primarily provide reference strains for all O, H, K, and F antigens, various toxins and other *E. coli* virulence factors.

Application

The reference strains are primarily sold as agar sticks concealed with paraffin, ensuring a long storage time until the first broaching.

Procedure

Strains from SSI Diagnostica can be used as positive and negative controls in different assays.

DEC PCR Kit

Traditionally, characterization of diarrhoeagenic *E. coli* (DEC) has been based on results from serotyping, followed by verocytotoxin cell assays. Therefore, strains have previously been overlooked where virulence genes have not had a serotype known for its pathogenicity.

The DEC Primer Kit makes it possible to more precisely identify pathogenic *E. coli* strains based on virulence genes and not only on the serotype. The kit identifies the following 7 virulence genes: intimin, verocytotoxin 1, verocytotoxin 2, heat stable enterotoxin (both human and porcine), heat labile enterotoxin, and the invasive plasmid antigen. 16S rDNA is used as internal control(2).

Among the different groups of intestinal pathogenic strains, the most important groups of DEC are:

- Verocytotoxin producing *E. coli* (VTEC)
- Attaching and effacing *E. coli* (A/EEC)
- Enteropathogenic *E. coli* (EPEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)

The DEC PCR Kit will allow determination of the virulence genes for all of these groups (see table 5).

Gene	*E. coli* group
Intimin (*eae*)	EPEC & A/EEC
Verocytotoxin 1 (*vtx1*)	VTEC
Verocytotoxin 2 (*vtx2*)	VTEC
Heat stable enterotoxin (*estA*-human)	ETEC
Heat stable enterotoxin (*estA*-porcine)	ETEC
Heat labile enterotoxin (*eltA*)	ETEC
Invasive plasmid antigen (*ipaH*)	ETEC
Internal control (16S rDNA)*	-

* Amplifies a fragment from most Gram-negative bacteria allowing an evaluation of the PCR.

Table 3. Virulence genes for groups in DEC PCR Kit.

Application

The DEC PCR Kit contains DEC primer mix, two PCR positive DNA controls, PCR ReadyMix (including loading buffer), TE-buffer and 10% Chelex-100. The kit contains reagents enough to perform 100 PCR tests.

Procedure

The DNA template is prepared by boiling and dilution of *E. coli* colonies from a selective medium. When an *E. coli* is tested positive by PCR, the strain should be recovered from the plate and serotyped by traditional methods.

GENEREL INFORMATION

Quality

The antiserum production is certified according to ISO 13485, and all antisera are CE marked except for *E. coli* K antisera.

Storage and shelf life

Antisera should be kept in a dark place at 2-8 °C. Shelf life is 4 years and the expiry date is minimum 2 years from date of delivery.

In the DEC PCR Kit the PCR ReadyMix, the DEC primer mix and the DNA controls should be stored at -20 °C. If necessary, dispense the reagents into several aliquots and store them at -20 °C. The DEC primer mix and the controls in use can be stored at 4 - 8 °C for up to 2 weeks. Store 10 % Chelex-100 and TE-buffer at room temperature.

Bacteriophages should be stored at 2-8 °C in a dark place. Expiry date is printed on the package.

Strains can be stored at room temperature. Once opened, the strain must be used immediately.

Reference

1. Ørskov et al., Bacteriol Rev. 1977 September; 41(3):667-710.
2. Persson, S., Olsen, K. E. P., Scheutz, F., Krogfelt, K. A. Gerner-Smidt, P. 2007. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clin Microbiol Infect. 13:516-524.

Information and ordering

For ordering please visit our webshop shop.ssidiagnostica.com, or contact one of our distributors listed on ssidiagnostica.com.

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