Comparing novel approaches for the rapid and specific detection of pathogenic *Escherichia coli* O157:H7 in food samples

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Abstract

In this review, a comparison has been made between conventional techniques and novel techniques for the detection of *Escherichia coli* O157:H7 in food samples. Food born- illnesses caused by *E. coli* O157:H7 is formed as a main global problem. Because the ingestion of a few shiga-like toxin producing *E. coli* O157:H7 in food samples can induce diarrheal diseases and person-to-person spread occurs, rapid detection of this food-borne pathogen is required to prevent epidemical spread of diarrheal diseases. An insight in the *E. coli* O157:H7 pathogenic mechanism has been given to look for possible targets to detect *E. coli* O157:H7 like Stx-1 and Stx-2. Although Stx-1 is mainly used as a target to detect *E. coli* O157:H7, Stx-2 seems to be a more promising target. Conventional used methods are described and compared to see which is more rapid and specific for the detection of *E. coli* O157:H7 in food samples. At first, culturing methods are shown to be slow and unspecific, because these techniques need enrichment steps to grow bacteria. Secondly, PCR techniques can detect *stx1* and *stx2* in 10^3 CFU per PCR assay, but this number is still too low for the detection in food samples. Furthermore, PCR assays are time consuming due to necessarily enrichment steps. In contrast, immuno-based conventional methods, like latex agglutination test, Sandwich ELISA and immune magnetic separation are more specific to detect *E. coli* O157:H7 in food samples. However these techniques still consume a lot of time and use of antibodies are rather expensive. To reduce costs in immuno-based methods, egg yolk produced Ig-Y is seen as an inexpensive alternative. Therefore it is of importance to further clarify and compare new approaches with conventional methods for a more rapid and specific detection of food-borne *E. coli* O157:H7 with immune-based methods.

Firstly, nanoporous membrane-based impedimetric immunosensor (NMBI) is shown to be a promising new technique to detect *E. coli* O157:H7 with a detection limit of 83.7 CFU/ml in less than 1 hour. Secondly, surface plasmon resonance (SPR) could detect *E. coli* O157:H7 in 10^6 CU/mL in less than 2 hours. However, when using bacteriophages the detection limit was lowered to 10^3 CFU/mL. Thirdly, lateral flow immuno assay (LFIA) showed to be detecting *E. coli* O157 antigens, Stx-1 and Stx2 with a specificity of 1.14×10^3 CFU/mL in less than three hours. Because NMBI, SPR and LFIA do not need any pre-enrichment and labeling steps, these techniques are more applicable and rapid when comparing them with conventional techniques. Of these three methods, NMBI is considered as the most rapid and specific novel technique for the detection of *E. coli* O157:H7 in food samples. Therefore, NMBI is the best technique suitable for the rapid and specific detection of *E. coli* O157:H7 in food samples. Although NMBI is not as specific as some conventional used method, it still has a strong potential to be used in the field of *E.coli* O157:H7 detection. Nevertheless, further studies are necessarily to develop novel inexpensive *E. coli* O157:H7 specific antibodies to lower the detection limit of NMBI and reduce costs for the application in food safety procedures.

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Chapter 1. Introduction

1.1 Problems with food-borne pathogens

Food-borne diseases (FBDs) caused by food-borne pathogens are a growing threat for the public health and food safety worldwide. The United States (U.S.) food and drug association estimates that 48 million cases of FBDs occur annually, with 128,000 hospitalized patients and 3,000 death occurrences in the U.S. (http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm103263.htm). FBDs are caused by pathogenic bacteria, enterotoxins and viruses established in consumable food and drinking supplies, for example, unpasteurized milk, undercooked meat products, unwashed fruits and contaminated water[1]. Common FBDs are diarrheal diseases (DDs) which are the second leading cause of death under five year old children. According to the World Health Organisation, DDs are responsible for approximately 760,000 deaths and 1.7 billion diagnosed patients worldwide (http://www.who.int/mediacentre/factsheets/fs330/en/)[2]. Several forms of food-borne DDs are known including bloody diarrhoea, hemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS) caused by enterohaemorrhagic shiga-like toxin producing *Escherichia coli* (STEC). Because the ingestion of a few STEC can induce DDs and person-to-person spread occurs[3,4], rapid detection of food-borne STEC is required to prevent epidemical spread of DDs.

1.2 Traditional methods to detect E. coli O157:H7

One of the most common cause of HC STEC strain screened for is *E. coli* O157:H7[5]. *E. coli* O157:H7 was first isolated in 1982 when 47 people developed bloody diarrhoea after eating STEC contaminated hamburgers in Michigan and Oregon[6]. Main causes of HC development are the shiga-toxins produced by *E. coli* O157:H7[7]. In the U.S., the economic costs of *E. coli* O157:H7 have been estimated at 405 million dollars annually. These costs include medical care, lost productivity and premature death[8]. Techniques to screen consumable food and drinking supplies for these bacteria are established to reduce these costs and prevent diagnosis of STEC infected patients. Although it is known that shiga-like toxin (Stx) induce HC and HUS, the pathogenic mechanism of Stx is still unclear. Nowadays several methods are used to screen for pathogenic STEC strains in raw food and liquids. Stx is a common used target to detect *E. coli* O157:H7, but lacks specificity. Conventional used screening methods for *E. coli* O157:H7 are culture based methods, polymerase chain reaction (PCR) methods and immunoassays[9]. Although these conventional methods are broadly used to detect pathogenic bacteria, the techniques are rather slow, expensive and need improvement in specificity to rapidly detect pathogenic *E. coli* O157:H7.

Therefore, in this thesis conventional detection techniques are compared with novel immune labeling techniques selected for the rapid and efficient detection of the pathogenic enterohaemorrhagic strain *E. coli* O157:H7.

To this end, the following research questions have been formulated:

1. What is known about the pathogenic mechanism used by E. coli O157:H7 produced shiga-toxins for causing FBDs?

2. Which conventional methods are used nowadays for the detection of pathogenic E. coli O157:H7?

3. Are there new methods based on immune labelling for detecting E. coli O157:H7 which are specific and rapid?

4. Which technique is best suitable for further use in the detection of E. coli O157:H7

Chapter 2. Pathogenic mechanism and virulence factors of STEC *O157:H7*

The pathogenesis of *E. coli* O157:H7 is similar to enteropathogenic *E. coli* strains in a way that they enter the intestines by passing through the environment of the stomach and destruct microvilli in the small intestine[10]. In contrast, *E. coli* O157:H7 strains produce Stx-1 and Stx-2. The pathogenesis of these Stxs are not completely understood, but it is known that they play a major role in the induction of HC and HUS. In this chapter, the pathogenesis of *E. coli* O157:H7 and the structure of Stx1 and Stx2 will be described to gain better knowledge of the virulence factors specifics and to see which virulence factors are good targets for the detection of *E. coli* O157:H7.

2.1 Pathogenesis of *E. coli* O157:H7

Similar for many *E. coli* strains, *E. coli* O157:H7 have a high acid and bile resistance. Once ingested, *E. coli* O157:H7 survive passage through the stomach and small intestine, and can colonize in both places[11]. Adherence to intestinal epithelial cells by *E. coli* O157:H7 inducing DDs is known as attaching and effacing lesions (AE lesions). AE lesions are characterized by destruction of intestinal microvilli, intimate association of bacteria with the host cells and accumulation of polymerized actin and other cytoskeletal components beneath the adherent bacteria[12] (Fig.1). Subsequently, stages of attachment are regulated by certain genes encoded on the locus of enterocyte effacement pathogenicity island. This island consists of 40 genes encoding virulence factors which are responsible for the attachment and destruction of the host cell surface[12]. Important virulence factors are intimin, translocated intimine receptor (Tir) and *E. coli* secreted F-like protein from prophage U (EspFu), a Tir cytoskeleton coupling protein[13]. When STEC is close to the epithelial cells, EspFu is secreted with Tir into the host cell by type 3 secretion system (T3SS). Tir and EspFu work cooperatively to subvert host cytoskeleton and actin polymerization. Insulin receptor tyrosine kinase substrate p53 (IRSp53) and actin nucleation-promoting factor Wiskott-Aldrich syndrome protein (N-WASP) are recruited by EspFu. These proteins are important regulators for actin cytoskeleton reorganization[13,14]. Finally, *E. coli* O157:H7 is tightly embedded in the intestinal epithelia where it inhibits the normal function of the gut, causing HC and HUS by secreting Stx1 and Stx2 which disrupt protein synthesis[15].

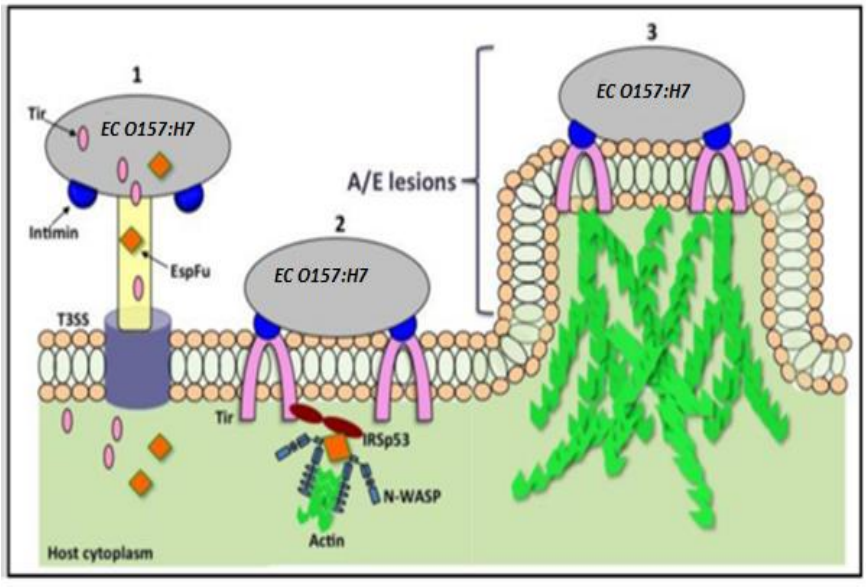

Figure 1: Schematic diagram of the formation of attaching and efficacing (A/E) lesions 1) *E. coli* O157:H7 injects Tir and EspFu into the host cytoplasm through the T3SS. 2) Intimate attaching of *E. coli* O157:H7 to the cell where Tir locates to the host-membrane and binds to intimin. Tir and EspFu recruit host factors like IRSp53 and N-WASP subvert host cytoskeleton and actin polymerization. 3) Host cytoskeleton subvention and actin polymerization results in the embedding of *E. coli* O157:H7 in intestinal epithelial cells. Source: *Nguyen, Y et al (2012)*

2.2 Stx-2 as a potential target for detecting *E. coli* O157:H7

Verotoxin, a shiga-like toxin, produced by *E. coli* O157:H7 was first described in 1977 by Konowalchuk et al. who detected cultured STEC strains producing an irreversible cytopathic effect on Vero cells. Most of these cultured strains were from infants with diarrhoea, suggesting a possible role of shigatoxin in the genesis of DDs[16]. Although seven *E. coli* strains are known of producing shiga-like toxins, *E. coli* O157:H7 is the most frequent common strain causing DDs[7]. In 1983, O'Brien and LaVeck purified verotoxin 1 and 2 from Konowalchuk's verotoxin producing *E. coli* strains and noted that verotoxin type 1 was antigenically and structurally similar to Stx-1 produced by *Shigella dysenteriae*, while verotoxin type 2 showed 60% homology with Stx-2[17]. Both toxins have one A subunit and five B subunits and are known as AB_5 ribosome-inactivating holotoxins with one enzymatically-active A subunit attached onto five binding B subunits forming a pentameric ring (Fig 2AB)[18]. Structurally, the active binding site of Stx-1 is blocked by part of the polypeptide chain of the A subunit, which makes Stx-1 less accessible. Because Stx-2 is more accessible than Stx-1, the pathogenicity of Stx-2 is much higher[19,20] (Fig 2CD). While many techniques are based on the detection of Stx-1, Stx-2 is more accessible and thus a better target for the detection of *E. coli* O157:H7.

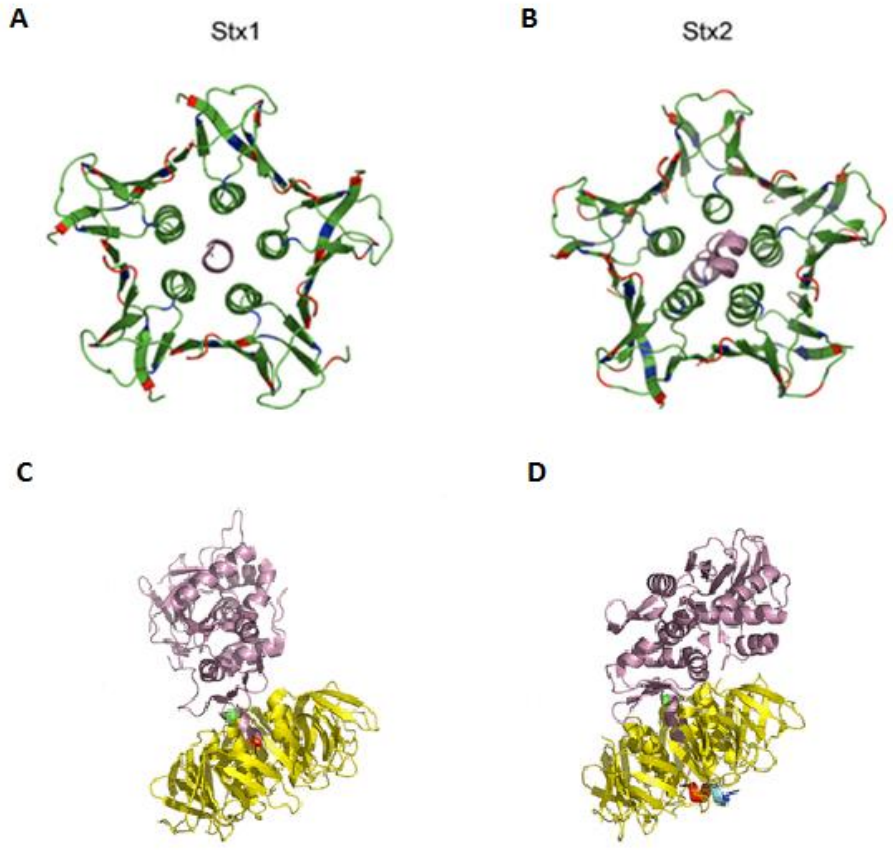

Figure 2: Crystal structure of Stx-1 and Stx-2. AB) view from above where the B-subunits forming a pentameric ring(green) with the A subunit in the middle (purple). C) side view of Stx-1 with the A-subunit (purple and its binding site (red) surrounded by the B-subunit pentameric ring (yellow). D) side view of Stx-2 with the A-subunit (purple and its binding site (red) surrounded by the B-subunit pentameric ring (yellow). The C-terminal end of Stx-2 A subunit extends well through the pore and ends below the base of the B-pentamer (D), in contrast to Stx1 A subunit (C). Source*: Kymre, L et al. (2015)*

Chapter 3 Established detection techniques for *E. coli O157:H7* in food

As mentioned before, there are several techniques already commonly used to detect *E. coli* O157:H7. In this chapter these established conventional methods are described, like culturing based methods, PCR, and different kinds of immunoassays, and the disadvantages and advantages of these techniques are highlighted.

3.1 Culture based method

It is known that *E. coli* O157:H7 ferment sorbitol either slowly over a period of days or not at all. Moreover, *E. coli* O157:H7 are found to produce verotoxin, a shiga-like toxin [21,22]. Culturing stool samples on sorbitol MacKonkey agar plates with testing for verotoxin production successfully screens *E. coli* O157:H7. Unfortunately, this culturing is done after STEC infection and screens for 10^4 colony forming unit (CFU) *E. coli* O157:H7 per gram faeces[23]. Because low concentration of *E. coli* O157:H7 can induce DDs, culturing methods are not useful to screen for *E. coli* O157:H7 in food[4]. Subsequently, culturing methods need more than 48 hours to culture *E.coli* O157:H7 and specifics are difficult to distinguish between strains[21–23]. Therefore more specific and rapid screening assays are important to prevent STEC infection by *E. coli* O157:H7.

3.2 Polymerase chain reaction

Polymerase chain reaction (PCR) is a nucleic acid amplification technique based on the isolation, amplification and quantification of a targeted genetic short DNA sequence and widely used for serotyping pathogenic bacterial, since the development by Mullis et al (1986)[24]. Moreover, PCR includes DNA polymerase, template DNA from the targeted pathogenic bacteria and two oligonucleotide primers to amplify the template DNA sequence[25]. In principle, PCR is used to amplify copies of a piece of targeted DNA resulting in thousands to million copies of this DNA sequence. For the detection of *E. coli* O157:H7, *stx1* and *stx2* genes coding for Stx-1 and Stx-2 are main targets for PCR[26]. Although PCR needs approximately 4 hours to get specific results, it is less time consuming than culture based methods. Furthermore, PCR brings expensive equipment and associated skills[9], which makes the use of PCR economically less attractive. Moreover, *stx1* and *stx2* are not specific enough to detect *E. coli* O157:H7 with PCR. Because PCR has a sensitivity of 10^3 CFU per PCR assay, the assay needs to be more specific because low numbers of *E. coli* O157:H7 can induce DDs[27]. Therefore, optimization of this technique and the development of novel probes are of great importance to get specific results with PCR technique.

3.3 Immunoassays

Immunoassays like enzyme-linked immunosorbent assay (ELISA), latex agglutination test (LAT) and immune magnetic separation (IMS) techniques are more rapid techniques to detect *E. coli* O157:H7[9]. By using specific antibodies with high affinity for Stx-1 and Stx-2 markers or outer membrane *E. coli* O157:H7 specific proteins, immunoassays can rapidly and specifically detect low concentrations of STEC in foodsamples[28].

3.3.1 Sandwich ELISA

ELISA is an immune assay which is widely used for the rapid screening of food-borne pathogenic bacteria[9]. Different kinds of ELISA methods are available, but the main type used for food screening is sandwich based ELISA. In principle, food samples are added onto rabbit polyclonal antibody-coated well specific for *E. coli* O157:H7. Furthermore, a monoclonal antibody is used as a detector antibody with a conjugated fluorescent label. When *E. coli* O157:H7 is present in food samples, more fluorescent labelling takes place and a higher optical density (OD) can be measured with a microplate reader[29]. With the sandwich ELISA method low concentrations of *E. coli* O157:H7 can be measured as low as 0.1 CFU per gram of ground poultry meat or mL of pasteurized milk[29]. After approximately 4 hours, sandwich ELISA can specifically detect *E. coli* O157:H7. However, mainly used mammalian polyclonal and monoclonal antibodies are expensive[9].

For this reason, Sunwoo et al. (2009) developed a sandwich ELISA using chicken anti- *E. coli* O157:H7 Ig-Y as the capture antibody and anti-*E. coli* O157 mouse mAb conjugated with biotin as the detection antibody[9]. Since Ig-Y antibodies are gain from egg yolk in large quantities and high titers, it reduces the costs compared with mainly used mammalian polyclonal and monoclonal antibodies[9]. Sunwoo et al. (2009) showed that using chicken anti-*E. coli* O157:H7 Ig-Y in sandwich ELISA detects *E. coli* O157:H7 specifically with a detection limit of 40 CFU/mL (Fig 3)[9]. Therefore, sandwich ELISA using egg yolk gained Ig-Y antibodies is a specific, sustainable and rapid detection method to detect *E. coli* O157:H7 in food or water.

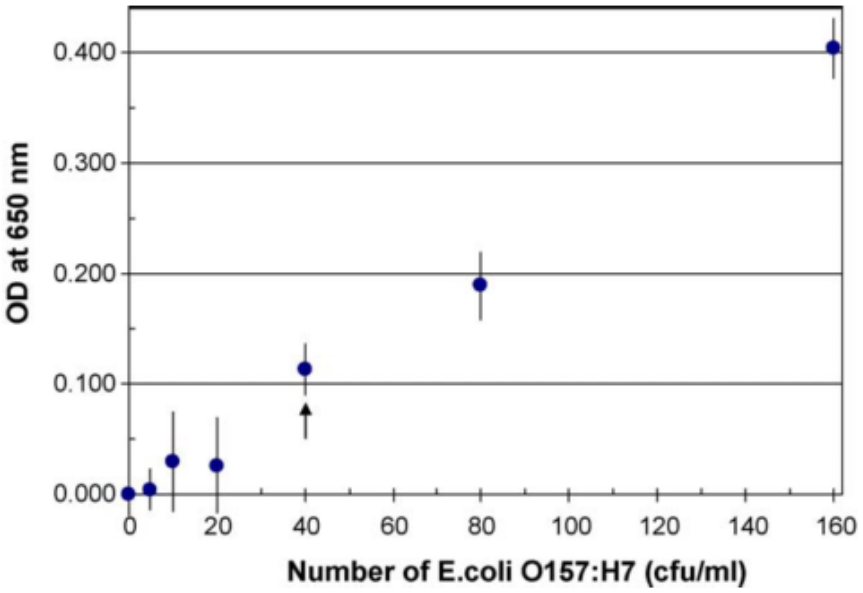

***Figure 3* Sandwich ELISA using IgY as capturing antibody** Sandwich ELISA was performed in 10 replicates. Vertical bar indicates standard error and arrow indicates the significant difference ($p<0.01$) between sample and control which is zero cells. Source: *Sunwoo, H. et al. (2009)*

3.3.2 Latex agglutination test

The latex agglutination test as another immune based assay can be used to rapidly detect *E. coli* O157:H7 in processed food samples[30,31]. Briefly, specific rabbit antibody reactive with *E. coli* O157 antigen are coated on latex beads. When processed food samples are placed on the latex beads, agglutination will be detected in the presence of *E. coli* O157 antigen. A control step is needed to rule out auto-agglutination, by using latex particles sensitized with pre-immune rabbit globulin[30].

In an earlier study, 25g beef was inoculated with *E. coli* O157:H7 strains (1,5 CFU/g) and showed 100% selectivity using LAT[30,31]. Although LAT is a rapid technique consuming less than 1 hour after the processing of food samples[30], it is best used in conjunction with sorbitol MacKonkey agar medium culturing which, as mentioned before, takes a lot of time[23,30]. Moreover, LAT is not specifically detecting the DDs causing *E. coli* O157:H7 pathogen. A sorbitol negative strain, *Escherichia hermannii*, agglutinates in *E. coli* serotype O157 antiserum as well[32]. Therefore, specifically *E. coli* O157:H7 serotyping is necessarily for definitive identification in food samples.

3.3.3 Immune magnetic separation

Since 1994, immune magnetic separation turns out to be a general technique to rapidly detect *E. coli* O157:H7 in food samples[33]. In short, magnetic beads conjugated with *E. coli* O157:H7 specific antibodies are added to a food sample solution. When antigen of *E. coli* O157:H7 is present, the magnetic

beads will bind to the antigen forming a magnetic bead-antigen complex. Using a strong magnet, the magnetic bead-antigen complexes will successfully separate the *E. coli* O157:H7 bacteria from the solution (Fig 4). Although low concentrations of *E. coli* O157:H7 strains give problems to detect pathogenic bacteria in food, IMS detects 20 CFU/ 10g beef, which is a high level of sensitivity[33]. In contrast, IMS does have non-specific binding sites on the magnetic beads which need to be reduced to enhance the specificity of IMS[34]. Moreover, IMS needs an assay time of approximately 24 hours, which needs to be less time consuming[9]. Therefore, novel approaches are needed to optimize IMS for *E. coli* O157:H7 screening in food samples.

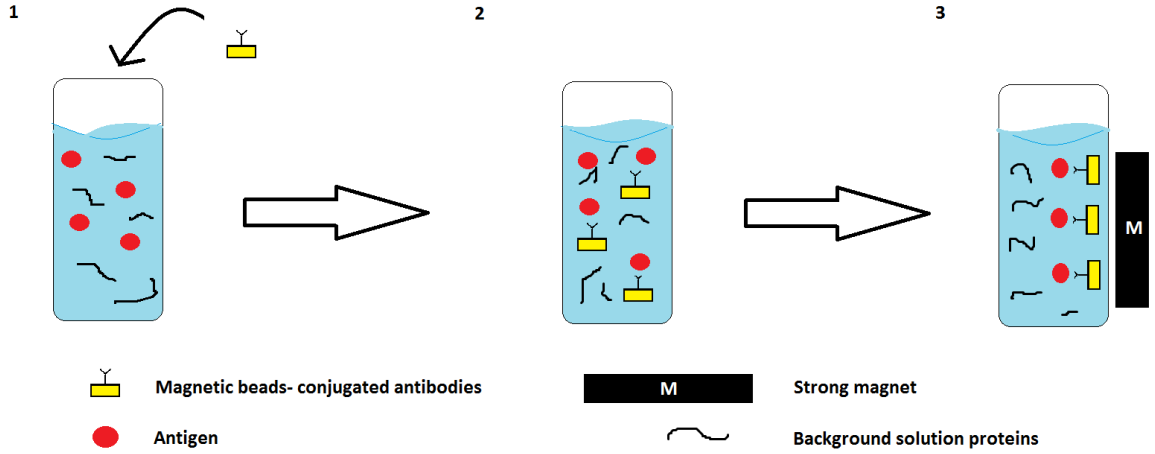

Figure 4: Schematic drawing of IMS technique. 1) Each 10 g portion of beef was dissolved into 90 ml of buffered peptone water supplemented with vancomycin 8 mg/l, cefixime 0 05 mg/l and cefsulodin 10 mg/l and incubated for 6 h at 37 ˚C. 2) Magnetic beads conjugated with target antigen specific antibodies were added to the solution to bind target antigen. 3) A strong magnetic separates the magnetic beads and the supernatant with background solution proteins were removed. Now the target antigen is separated and isolated from the food suspension[33,34].

3.4 Immune labelling techniques are rapid and specific

In this chapter, conventional methods used to detect *E. coli* O157:H7 are discussed to give an identification of factors which can be optimized. Comparing these conventional techniques (Table 1), immunoassays are seen as the best conventional method, because their rapid and more specific than PCR and culturing on sorbitol McKonkey agar plates. Sandwich ELISA are seen as the most specific technique for the detection of *E. coli* O157:H7, but this technique is widely used with expensive mammalian antibodies[9]. Using inexpensive egg yolk produced Ig-Y antibodies, a sustainable, specific sandwich ELISA technique is developed. However, to perform sandwich ELISA takes 4 hours and needs to be reduced. Likewise, IMS is rather specific as well, but still has an assay time of approximately 24 hours, which need to be reduced[9,33]. Because immune labelling is the best conventional technique for rapid and specific detection of food-borne *E. coli* O157:H7, new approaches to specifically detect food-borne *E. coli* O157:H7 are mainly immune-based.

Table 1: Overview of conventional techniques to detect *E. coli O157:H7*

Conventional Techniques	**target**	**Time consuming (hours)**	**Limit of detection**
Sorbitol McKonkey agar plate	***E. coli* O157:H7**	**>48**	**10^4 CFU/stool**
PCR	**stx-1 and stx-2**	**~ 4**	**10^3 CFU/assay**
Sandwich ELISA **- *mAB*** **- *Ig-Y***	***E. coli* O157:H7**	**~ 4**	 **- 0.1 CFU/g** **- 40 CFU/mL**
LAT	***E.coli* O157 *strains***	***<1**	**1.5 CFU/g**
IMS	***E. coli* O157:H7**	**~ 24**	**2 CFU/g**

* *= LAT is best used when combined with culturing on Sorbitol McKonkey agar plates, which consumes a lot more time.*

Chapter 4 Novel approaches in the detection of *E. coli* O157:H7

Novel techniques have been introduced for the detection of *E.coli* O157:H7. Many of these techniques are immune based methods, developed for the rapid and specific detection of food-born pathogenic bacteria, like *E. coli* O157:H7. Biosensors, which incorporate a bio receptor closely integrated within a physicochemical transducer or transducing microsystem, are proven to be a rapid, sensitive technique for the detection of *E. coli* O157:H7[35,36]. In this chapter, the advantages of three novel immune based techniques are highlighted and afterwards compared with earlier described conventional methods.

4.1 Nanoporous membrane-based impedimetric immunosensor

Nanoporous membrane-based impedimetric immunosensoring technique (NMBI) is a label-free specific technique for the applications in food and environmental safety[37]**.** NMBI can specifically detect *E. coli* O157:H7 in whole milk samples and food sample solutions without time consuming labelling processes[37]. Briefly, NMBI uses polydimethylsiloxane (PDMS) chambers to sandwich a nanoporous membrane with a uniform 100 nm porediameter. After treatment with hyaluronic acid (HA), anti-*E. coli* O157:H7 antibodies react with the membrane in the upper PDMS chamber and conjugates with the membrane surface. After an Ag/AgCL electrode is placed into the upper and lower PDMS chamber, the food sample solutions are added into the upper chamber. When *E. coli* O157:H7 is present in the sample and binds to the specific antibody, the ionic current flow will decrease and changes the impedance (Fig 5). Within 30 minutes electrochemical impedance spectroscopy (EIS), differences in magnitude of impedance, at a frequency ranging from 1 Hz to 100 kHz, can be analysed by the normalized impedance change (NIC) with respect to the control[37,38].

The NMBI in combination with EIS can detect *E. coli* O157:H7 in concentrations 83.7 CFU/ml raw milk specifically. Joung et al. (2013) already looked for the specificity of the *E. coli* O157:H7 antibody and concluded the specific detection of the target antigen[37]. Because the preparation of the PDMS sandwiched membrane with antibodies can be stored at 4˚C until further use, NMBI is a rapid technique which only needs sample addition to the membrane to get results[37]. Therefore, this label-free nanoporous sensor is rapid and significant for applications in food pathogen detection.

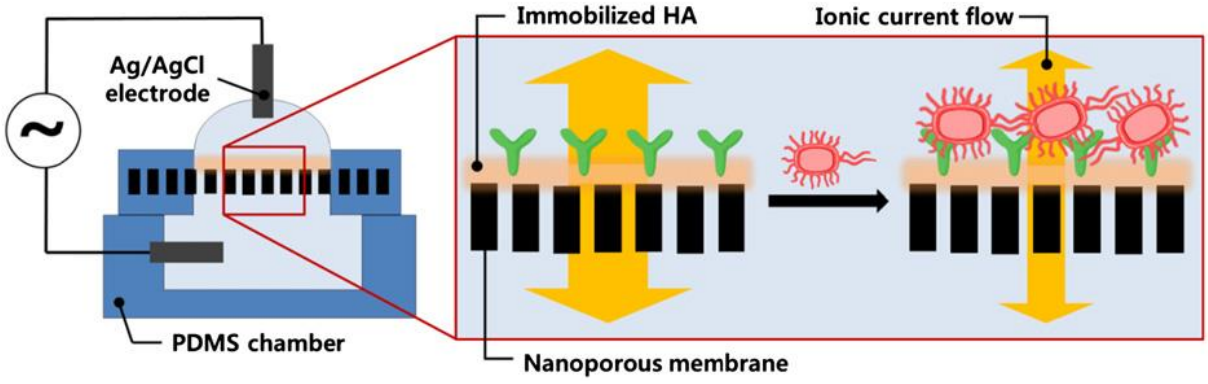

Figure 5: Schematic drawing of HA-coated nanoporous immunosensor for the detection of *E. coli* O157:H7. Electrodes inside the upper and lower PDMS chambers regulate the ionic current flow with a frequency ranging from 1Hz to 100kHz. The enlarged box shows the cross-section of the HA/antibody-modified membrane. The change of impedance is analyzed with EIS. Source: *C. Joung et al (2013)*

4.2 Surface plasmon resonance

Surface plasmon resonance (SPR) monitors antigen-antibody interactions based on the change in refractive index (RI) on a sensor[39]. In 1983, SPR was first introduced for the detection of gas and bio-sensing applications by Liedberg et al[40]. Nowadays, SPR is used for the detection of food-born pathogenic bacteria, such as *E. coli* O157:H7[5,39,41]. In principle, A light source passes light through a prism and reflects off the back site of a sensor chip surface into a detector. This sensor chip surface is created by a glass slide coated with a thin layer of gold film. On top of the sensor chip, a dextran matrix covering the gold film acts as a substrate to which antibodies can be attached. The light beam is absorbed by electrons in the middle of the sensor chip causing them to resonate. When the pathogen bacteria is detected and bound to the antibodies, the resonating electrons change and the light will reflect differently (Fig 6)[42]. The difference in changing resonating electrons are processed in to a graph (Fig 7) giving the change RI, which is different when *E. coli* O157:H7 is bound to the capture antibody.

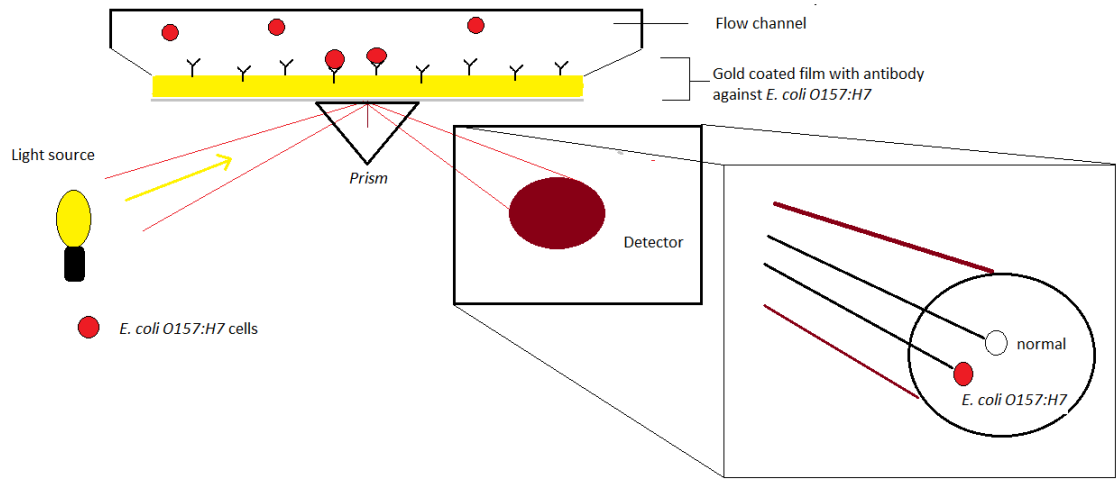

Figure 6: schematic drawing of the surface plasmon resonance technique. Food samples are flowing through the flow channel and *E. coli O157:H7,* if present, will bind to the antibodies attached to the golden sensor chip. When *E. coli O157:H7* are attached to the antibody, the change in electron resonance forms a shift in the refractive index detected by the detector.

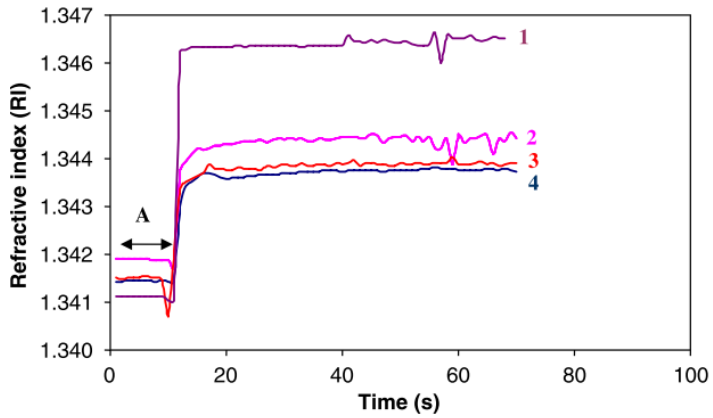

Figure 7: Results from an SPR detecting *E. coli* O157:H7 specifically. This figure gives an example to show the specific detection of *E. coli* O157:H7. 1) .*E. coli* O157:H7, *2) E. coli* K12, *3) Shigella sp*., 4) no bacteria. Source: J. Waswa et al. (2007)

Subramanian et al. (2006) found that the detection limit of SPR assay for *E. coli* O157:H7 detection was 10^6 CFU/ml in less than 2 hours[43]. In contrast, the main advantage of SPR is that it doesn't require any labelling steps, making it a more rapid technique than any conventional used method[41]. Although SPR is a simple technique and does not require a lot of labour time, it still needs more specific antibodies to lower the detection limit[41,43].

A recent study of Tawil et al. (2012) used bacteriophages instead of antibodies combined with SPR for the detection of *Staphylococcus aureus* and *E. coli* strains[44]. Advantages using bacteriophages are that they are able to distinguish between live and dead cells, easy to produce and cost effective[44]. Tawil et al. found that the detection limit of SPR using bacteriophages in detecting *E. coli* strains is reduced to 10^3 CFU/mL in 20 minutes[44]. Therefore, SPR detection using bacteriophages is a rapid novel detection technique to specifically detect *E. coli* O157:H7.

4.3 Lateral flow immunoassay

Recently, Wang et al. (2016) developed a lateral flow immunoassay (LFIA) for the detection of Stx-1 and Stx-2 or *E. coli* O157 antigen in food samples[45]. In principle, when Stx-1, Stx-2 or *E. coli* O157 antigen is present in food samples, colloidal gold-labelled antibody in a conjugation pad captures antigen. Moreover, the food sample migrates to a test line to generate a red signal. The red signal is due to the formation of antigen-antibody complexes in a nutrocellulose membrane, and when a high intensity of red signal is given, high concentrations of the target antigen is present in the food sample. Next to a food sample, a control group is added as well to validate results (Fig 8)[45].

Advantages of LFIA are that LFIA is rapid and specific. In 3 hours, *E.coli*, Stx-1 and Stx-2 can be detected within a detection limit of 1.14 x 10^3 CFU/mL[46]. Furthermore, LFIA is a sustainable, user friendly simple technique which needs low costs to be performed. Because highly toxic Stx-1 and Stx-2 can be present in food samples with *E. coli* O157 strains, LFIA can be used to detect both of these target antigens[45]. Therefore LFIA is a promising novel technique for the detection of pathogenic *E. coli* O157 strains.

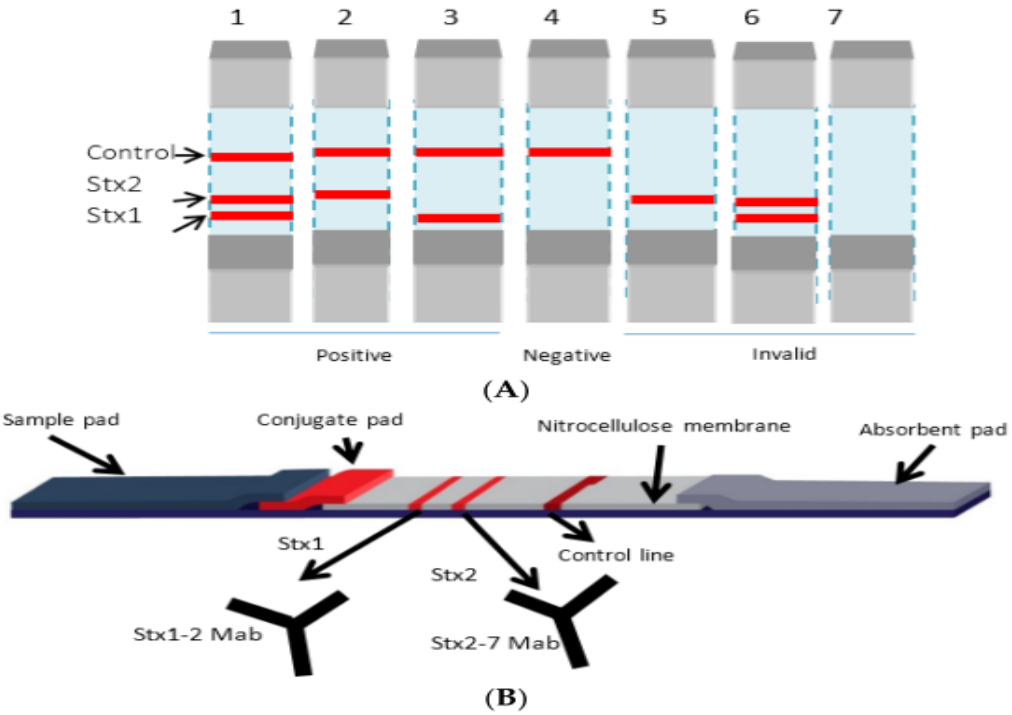

Figure 8*:* Diagrammatic representation of Lateral Flow Immunoassay Device. A) Expected results with a control group, Stx1 and Stx2. Instead of Stxs, *E. coli O157* can be detected as well. A negative result is when no other line as the control line is seen. A positive result is when control lines and Stx lines are present. Tests are invalid, if the control group does not show a red signal line. B) The LFIA device. Source: Wang et al (2016)

4.4 NMBI as a novel rapid and specific technique for *E.coli O157:H7 detection*

In this chapter an insight in three novel approaches for the detection of *E. coli* O157:H7 has been given. Because these novel techniques do not need any pre-enrichment and labeling steps, they are more user friendly and rapid when comparing them with conventional techniques (chapter 3). However, the use of expensive antibodies still needs inexpensive alternatives. Although all three techniques have shown to be rapid and specific (Table 2), NMBI is seen as the most rapid and specific novel technique for the detection of *E. coli* O157:H7 in food samples[37,38].

Tabel 2 **Overview of novel techniques to detect *E. coli O157:H7***

Novel techniques	**Target**	**Time consuming (h)**	**Limit of detection**
NMBI	***E. coli* O157:H7**	**< 1**	**83.7 CFU/ml**
SPR **- *Antibodies*** **- *Bacteriophage***	 **- *E. coli* O157:H7** **- *E. coli strains***	 **- < 2** **- < 1**	 **- 10^6 CFU/ml** **- 10^3 CFU/mL**
LFIA	**Stx-1, Stx-2, *E. coli* O157:H7**	**< 3**	**1.14 x 10^3 CFU/mL**

Discussion

At first, insight has been given in the pathogenic mechanism of *E. coli* O157:H7 and has given potential targets like Stx-1 and Stx-2 proteins to detect *E. coli* O157:H7. Although *stx1* and *stx2* can be detected in food samples by PCR, it is an expensive and time-consuming process. Moreover, the detection limit needs to be more specific. However, Stx-1 and Stx-2 are not the only proteins which causes DDs. Dellanoy et al. (2013) have found that targeting a combination of different EHEC genes like, *espK* with either *espV*, *ureD*,or *Z2098* with real time PCR, gives a more specific detection of EHEC strains, like *E. coli* O157:H7, compared to detecting for only *stx1* and *stx2*[7]. In contrast, conventional immuno-based methods like ELISA and LAT are more specific and rapid than PCR[9,30]. Therefore immuno-based methods are the best targets to approach with novel techniques.

Secondly, when comparing NMBI, SPR and LFIA for the specific and rapid detection of *E. coli* O157:H7 shows that NMBI is a more promising technique for further studies. Because LFIA has a detection limit of 1.14 x 10^3 CFU/mL, it still needs more inexpensive specific antibodies for the optimization of this technique. Furthermore, SRP capturing of *E. coli* O157:H7 has some limits to reduce the sensitivity of immuno-sensors as well. Firstly, only refractive index changes occurring within the 300 nm distance from the surface will cause a change in the SPR signal. *E. coli* O157:H7 are approximately 1 µm big, which only interact with the top of the immune sensor. Therefore only small concentrations produce a measureable signal, which decreases the sensitivity of detecting *E. coli* O157:H7 with direct SRP[41]. Secondly, due to the large size of *E. coli* O157:H7, direct cell binding requires a high cell-antibody binding affinity to withstand the shear force effect created by the laminar flow in the flow channel[47]. To overcome these limits, a subtractive inhibition assay in combination with SRP is developed to reduce the detection limit to 3.0×10^4 CFU/mL[41]. Moreover, replacing expensive antibodies with inexpensive bacteriophages can reduce the detection limit to 10^3 CFU/ml *E. coli* O157:H7in less than 20 minutes[44]. Although SPR optimization made this technique more specific, NMBI has a detection limit of 83.7 CFU/ml and can be performed within 1 hour[37,48]. Therefore NMBI is best suitable to detect *E. coli* O157:H7 rapidly and specific in food samples when comparing with SPR and LFIA.

Thirdly, when comparing NMBI with conventional used immuno-based methods, it shows that NMBI has a higher detection limit then ELISA, LAT and IMS (Table 3). However, NMBI is a more rapid technique which makes it a promising technique for the detection of *E. coli* O157:H7[37]. In contrast, immuno-based techniques are expensive due to the use of monoclonal antibodies. Egg yolk produced Ig-Y can be used to reduce costs in immuno-based techniques to detect *E. coli* O157:H7[9]. Future studies are necessarily to optimize NMBI with the use of egg yolk produced Ig-Y against *E. coli* O157:H7 to reduce costs and detection limit.

Table 3 Comparison of conventional immuno-based assays with NMBI

Techniques	**Target**	**Time consuming (h)**	**Limit of detection**
Sandwich ELISA	***E. coli O157:H7***	**~ 4**	**0.1 CFU/g**
LAT	***E.coli O157 strains***	***<1**	**1.5 CFU/g**
IMS	***E. coli O157:H7***	**~ 24**	**2 CFU/g**
NMBI	***E. coli O157:H7***	**<1**	**83.7 CFU/ml**

** = LAT is best used when combined with culturing on Sorbitol McKonkey agar plates, which consumes a lot more time.*

Conclusion

In conclusion, NMBI is the best technique suitable for the rapid and specific detection of *E. coli* O157:H7 in food samples. Although conventional used immuno-based assays are more specific, these techniques are still time consuming for the detection of *E. coli* and costly using expensive antibodies. Even though NMBI is not as specific as conventional used method, it still is rapidly able to be used in the field of *E.coli* O157:H7 detection. Further studies are necessarily to develop novel inexpensive *E. coli* O157:H7 specific antibodies to lower the detection limit of NMBI for the application in food safety procedures.

References

1. Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M. & Swerdlow, D. L. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* **11,** 603–609 (2005).

2. Sjöling, Ä., Sadeghipoorjahromi, L., Novak, D. & Tobias, J. Detection of major diarrheagenic bacterial pathogens by multiplex PCR panels. *Microbiol. Res.* **172,** 34–40 (2015).

3. Snedeker, K. G., Shaw, D. J., Locking, M. E., Prescott, R. J. & Prescott -RobinPrescott, R. J. Primary and secondary cases in *Escherichia coli* O157 outbreaks: a statistical analysis. *BMC Infect. Dis.* **9,** (2009).

4. Tilden, J. *et al.* A new route of transmission for *Escherichia coli*: Infection from dry fermented salami. *Am. J. Public Health* **86,** 1142–1145 (1996).

5. Wang, Y., Ye, Z., Si, C. & Ying, Y. Monitoring of *Escherichia coli* O157:H7 in food samples using lectin based surface plasmon resonance biosensor. *Food Chem.* **136,** 1303–1308 (2013).

6. Riley, L. W. *et al.* Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* **308,** 681–5 (1983).

7. Delannoy, S., Beutin, L. & Fach, P. Discrimination of Enterohemorrhagic *Escherichia coli* (EHEC) from Non-EHEC Strains Based on Detection of Various Combinations of Type III Effector Genes. *J. Clin. Microbiol. Microbiol.* **51,** 3257–3262 (2013).

8. Frenzen, P. D., Drake, A., Angulo, F. J. & Group, E. I. P. F. W. Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J. Food Prot.* **68,** 2623–2630 (2005).

9. Sunwoo, H. H., Wang, W. W. & Sim, J. S. Detection of *Escherichia coli* O157:H7 using chicken immunoglobulin Y. *Immunol. Lett.* **106,** 191–193 (2006).

10. Karmali, M. A. *et al.* Association of Genomic O Island 122 of *Escherichia coli* EDL 933 with Verocytotoxin-Producing *Escherichia coli* Seropathotypes That Are Linked to Epidemic and/or Serious Disease. *J. Clin. Microbiol.* **41,** 4930–4940 (2003).

11. Price, S. B., Wright, J. C., Degraves, F. J., Castanie-Cornet, M.-P. & Foster, J. W. Acid Resistance Systems Required for Survival of *Escherichia coli* O157:H7 in the Bovine Gastrointestinal Tract and in Apple Cider Are Different. *Appl. Environ. Microbiol.* **70,** 4792–4799 (2004).

12. Kanamaru, K., Tatsuno, I., Tobe, T. & Sasakawa, C. SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol. Microbiol.* **38,** 805–16 (2000).

13. Weiss, S. M. *et al.* IRSp53 Links the Enterohemorrhagic *E. coli* Effectors Tir and EspFU for Actin Pedestal Formation. *Cell Host Microbe* **5,** 244–258 (2009).

14. Campellone, K. G., Robbins, D. & Leong, J. M. EspF U Is a Translocated EHEC Effector that Interacts with Tir and N-WASP and Promotes Nck-Independent Actin Assembly. *Dev. Cell* **7,** 217–228 (2004).

15. Endo, Y. *et al.* Site of action of a Vero toxin (VT2) from *Escherichia coli* O157 : H7 and of Shiga toxin on eukaryotic ribosomes. *Eur. J. Biochem.* **171,** 45–50 (1988).

16. Konowalchuk, J., Speirs, J. I. & Stavric, S. Vero Response to a Cytotoxm of *Escherichia coli*. **18,** 775–779 (1977).

17. O ’brien, A. D. & Laveckt, G. D. Purification and Characterization of a *Shigella dysenteriae* 1-Like Toxin Produced by *Escherichia coli*. *Infect. Immun.* **40,** 675–683 (1983).

18. Bunger, J., Melton-Celsa, A., Maynard, E. & O'Brien, A. D. Reduced Toxicity of Shiga Toxin (Stx) Type 2c in Mice Compared to Stx2d Is Associated with Instability of Stx2c Holotoxin. *Toxins (Basel).* **7,** 2306–2320 (2015).

19. Fraser, M. E. *et al.* Structure of Shiga Toxin Type 2 (Stx2) from *Escherichia coli* O157:H7*. *J. Biol. Chem.* **279,** 27511–27517 (2004).

20. Kymre, L., Simm, R., Skotland, T. & Sandvig, K. Different roles of the C-terminal end of Stx1A and Stx2A for AB 5 complex integrity and retrograde transport of Stx in HeLa cells. *FEMS Pathog. Dis.* **73,** (2015).

21. Wells, J. G. *et al.* Laboratory Investigation of Hemorrhagic Colitis Outbreaks Associated with a Rare *Escherichia coli* Serotype. *J. Clin. Microbiol.* **18,** 512–520 (1983).

22. Remis, R. S. *et al.* Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. *Ann. Intern. Med.* **101,** 624–6 (1984).

23. March, S. B. & Ratnam, S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* **23,** 869–872 (1986).

24. Cheng, K. *et al.* A Comparative Study of Traditional Flagella Serotyping and Liquid Chromatography-Tandem Mass Spectrometry Based Flagella Typing (MS-H) on Clinical *Escherichia coli* Isolates. *J. Clin. Microbiol.* **52,** 2275–2278 (2014).

25. Mullis, K. *et al.* Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harb. Symp. Quant. Biol.* **LI.,** 263–273 (1986).

26. Moyne, A. L., Harris, L. J. & Marco, M. L. Assessments of Total and Viable *Escherichia coli* O157:H7 on Field and Laboratory Grown Lettuce. *PLoS One* **8,** 1–5 (2013).

27. Karmali, M. A., Gannon, V. & Sargeant, J. M. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet. Microbiol.* **140,** 360–370 (2010).

28. Mondani, L. *et al.* Fast detection of both O157 and non-O157 shiga-toxin producing *Escherichia coli* by real-time optical immunoassay. *Lett. Appl. Microbiol.* **62,** 39–46 (2016).

29. Zhao, Z.-J. & Liu, X.-M. Preparation of Monoclonal Antibody and Development of Enzyme-linked Immunosorbent Assay Specific for *Escherichia coli* O157 in Foods. *Biomed. Environ. Sci.* **18,** 254–259 (2005).

30. March, S. B. & Ratnam, S. Latex Agglutination Test for Detection of *Escherichia coli* Serotype O157. *J. Clin. Microbiol.* **27,** 1675–1677 (1989).

31. Doyle, M. P. & Schoeni, J. L. Isolation of *Escherichia coli* O157:H7 from Retail Fresh Meats and Poultry. *Appl. Environ. Microbiol.* **53,** 2394–2396 (1987).

32. Lior, H. & Borczyk, A. A. False positive identification of *Escherichia coli* O157. *Lancet* **329,** 333 (1987).

33. Wright, D. J., Chapman, P. A. & Siddons, C. A. Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiol. Infect* **113,** 31–39 (1994).

34. Fratamico, P. M., Schultz, F. J. & Buchanan, R. L. Rapid isolation of *Escherichia coli* O157:H7 from enrichment cultures of foods using an immunomagnetic separation method. *Food Microbiol.* **9,** 105–13 (1992).

35. Lazcka, O., Campo, F. J. Del & Munoz, F. X. Pathogen detection: A perspective of traditional methods and biosensors. *Biosens. Bioelectron.* **22,** 1205–1217 (2007).

36. Ivnitski, D., Abdel-Hamid, I., Atanasov, P., Wilkins, E. & Stricker, S. Application of

Electrochemical Biosensors for Detection of Food Pathogenic Bacteria. *Electroanalysis* **12,** 317–325 (2000).

37. Joung, C. K. *et al.* A nanoporous membrane-based impedimetric immunosensor for label-free detection of pathogenic bacteria in whole milk. *Biosens. Bioelectron.* **44,** 210–215 (2013).

38. Xu, M., Wang, R. & Li, Y. Rapid detection of *Escherichia coli* O157:H7 and *Salmonella Typhimurium* in foods using an electrochemical immunosensor based on screen-printed interdigitated microelectrode and immunomagnetic separation. *Talanta* **148,** 200–208 (2016).

39. Waswa, J., Irudayaraj, J. & DebRoy, C. Direct detection of *E. Coli* O157:H7 in selected food systems by a surface plasmon resonance biosensor. *LWT - Food Science and Technology* **40,** 187–192 (2007).

40. Liedberg, B., Nylander, C. & Lunström, I. Surface plasmon resonance for gas detection and biosensing. *Sensors and Actuators* **4,** 299–304 (1983).

41. Wang, Y., Ye, Z., Si, C. & Ying, Y. Subtractive inhibition assay for the detection of *E. coli* O157:H7 using surface plasmon resonance. *Sensors* **11,** 2728–2739 (2011).

42. Vashist, S. K. A highly-sensitive and rapid Surface Plasmon Resonance immunoassay procedure based on the covalent- orientated immobilization of antibodies. *Protoc. Exch.* 1–21 (2012).

43. Subramanian, A., Irudayaraj, J. & Ryan, T. A mixed self-assembled monolayer-based surface plasmon immunosensor for detection of *E. coli* O157:H7. *Biosens. Bioelectron.* **21,** 998–1006 (2006).

44. Tawil, N., Sacher, E., Mandeville, R. & Meunier, M. Surface plasmon resonance detection of *E. coli* and methicillin-resistant *S. aureus* using bacteriophages. *Biosens. Bioelectron.* **37,** 24–29 (2012).

45. Wang, J. *et al.* Rapid Detection of *Escherichia coli* O157 and Shiga Toxins by Lateral Flow Immunoassays. *Toxins (Basel).* **8,** 92 (2016).

46. Chen, M. *et al.* Dual gold nanoparticle lateflow immunoassay for sensitive detection of *Escherichia coli* O157:H7. *Anal. Chim. Acta* **876,** 71–76 (2015).

47. Skottrup, P. D., Nicolaisen, M. & Fejer Justesen, A. Towards on-site pathogen detection using antibody-based sensors. *Biosens. Bioelectron.* **24,** 339–348 (2008).

48. Tang, Y., Li, Z., Luo, Q., Liu, J. & Wu, J. Bacteria detection based on its blockage effect on silicon nanopore array. *Biosens. Bioelectron.* **79,** 715–720 (2016).