Methods for Detecting Enterohaemorrhagic *Escherichia Coli* in Food

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Enterohaemorrhagic Escherichia coli (EHEC) serogroup determines worldwide foodborne illnesses and remains one of the major concerns for the population and for the food industry. These strains, indeed, determine gastrointestinal disease varying from diarrhoea to haemorrhagic colitis, haemolytic uraemic syndrome, and thrombotic thrombocytopaenic purpura. Classic detection methods are based on specific enrichment, often coupled with immunomagnetic separation system, specific media, and different immunoassays. Molecular detection methods, based on DNA probes and PCR, are used to detect the virulence genes and the specific genes associated to the serogroups. Most of the research is focused on E. coli O157:H7, for which the validated standard method ISO 16654/2001 is applied. Regarding the methods used for E. coli non-O157, it is interesting to highlight the existence of the draft of the European Committee for Standardization, which in the near future might constitute the first standard for non-O157 EHEC. This paper describes the state-of-the-art laboratory methods and commercial kits for detecting strains of EHEC vehiculated with foods.

Keywords enterohaemorrhagic *Escherichia coli*, classic and molecular methods, commercial kits

Introduction

Among worldwide outbreaks of foodborne illness caused by pathogen microorganisms, the most worrying are related to enterohaemorrhagic *Escherichia coli* (EHEC). This serogroup is a subset both of verocytotoxigenic or Shiga toxin-producing *E. coli* – since they produce verocytotoxins that are active on Vero cells, similar to the toxin produced by *Shigella dysenteriae* – and of enteropathogenic *E. coli* since they are able to form attaching and effacing lesions on epithelial cells.
The worry is justified both for their serious symptoms – gastrointestinal disease varying from diarrhoea to haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopaenic purpura(1,2) – and an increasingly broad range of food identified as vehicles of foodborne illness.(3) In fact, beside minced beef, other foods related to EHEC include yoghurt,(4) cooked maize,(5) mayonnaise,(6) salami,(7) cheese,(8) alfalfa sprouts,(9) lettuce,(10) radish sprouts,(11) raw milk,(12) and retail minced beef.(13) Moreover, the EHEC are characterized by extremely low infective doses; studies on *E. coli* O157:H7 reported infective dose values lower than 100 cfu(14,15) and between 20 and 700 cfu.(16)
Among the EHEC, the serotype O157:H7 is found in food more often than the non-O157 and, therefore, more frequently associated with foodborne illnesses. Worldwide food research is mainly focused on this serotype. However, the role of the *E. coli* non-O157 is more important than it was believed until now. They include O26, O103, O111, O118, and O145 serogroups,(17) and also O55 and O91 serogroups.(18)
Nataro and Kaper(1) suggest that 20 to 50% of EHEC infections are caused by non-O157 serotypes. According to several studies, both the O26 and O111 serogroups have been frequently associated with worldwide foodborne illnesses.(19–21) Due to those works highlighting the lack of standard methods to detect *E. coli* non-O157 in food,(22–24) the European Food Safety Authority has recently proposed harmonized technical specifications based on scientific opinion of the Panel of Biological Hazard.(25) Moreover, the European Committee for Standardization/Comité Européen de Normalisation (CEN) is currently developing standard methods for detecting serogroups O26, O103, O111, and O145 in foodstuffs.(25) Beside culture, biochemical, and immunological methods, the molecular methods are increasingly used due to their powerful specificity and sensitivity. Methods available to detect specifically *E. coli* O157 and O157:H7 have been reviewed,(26–28) as have methods for the *E. coli* O26 serogroup.(24) This paper describes state-of-art laboratory and standard methods, together with commercial kits for detecting EHEC vehiculated with food.

Classic Methods

Classic methods offer a simple and cheap way to detect pathogens in food; however, they are less sensitive and focused than molecular methods. The most widely used of these methods employs selective culture enrichment followed by the use of specific antibodies.

Culture Media

Culture methods to detect *E. coli* O157:H7 are based on phenotypic characteristics, such as: the inability to ferment sorbitol, to hydrolyze 4-methylumbrelliferyl-D-glucuronide due to the lack of β-glucuronidase enzyme, and to grow at temperature > 44°C.(29) Disadvantages of these methods are related to the low infectious dose, the presence of atypical strains that can ferment sorbitol, and to the time required to obtain results (Table 1).
Concerning the low infectious dose in food, improving recovery enrichment in broth is necessary before plating on solid media. Currently, the immunomagnetic separation system (IMS), which consists of paramagnetic polystyrene

microspheres coated with specific antibodies, is used for better recovery.(30–32) The IMS method has the advantage of combining a reduction in detection time with increased sensitivity due to the pre-concentration effect. On the other hand, cross-reactivity with other bacteria, such as *E. hermani*, *Salmonella* O30 group, *Hafnia alvei*, and *Citrobacter freundii*,(33) is the main shortcoming. Testing the usefulness of IMS for detecting EHEC in vegetables, Šafǎriková and Šafǎrik(31) reported that after IMS of *E. coli* O111 and *E. coli* O157, the percentage of colonies found positive was higher – 93% and 100%, respectively – than those obtained using direct plating – 36–40% and 53%, respectively. Other authors(34) reported that IMS was a weak tool in detecting O26 and O111 serotypes compared to other techniques such as those based on Polymerase Chain Reaction (PCR).

The usual enrichment media for *E. coli* O157 are modified trypticase-soy broth (mTSB) with novobiocin, acriflavin, or cefixime, cefsulodin, and vancomycin,(35) modified *E. coli* broth with novobiocin (mEC+n), and buffered peptone water (BPW) supplemented with vancomycin, cefsulodin, and cefixime.(35,36)

A good selective solid media is the Sorbitol Mac-Conkey Agar (SMAC) that allows the identification of *E. coli* O157 since their colonies remain colorless due to the lack of sorbitol fermentation. To increase selectivity, the SMAC is modified adding rhamnose and cefixime or cefixime and potassium tellurite (CT-SMAC).(26,35,36) These media do not allow the identification of sorbitol fermenting *E. coli* O157; in fact, they grow in them as colored colonies. Therefore, taking advantage of the lack of the *β*-glucuronidase enzyme, the fluorogenic substrate 4-methylumbelliferyl *β*-D-glucuronide and the chromogenic substrate 5-bromo-4-chloro-3-indoxyl-*β*-D-glucuronide are added to the media.(26,37) These two substrates work well, although their high cost could restrict their use in laboratories.(26)

Another, more specific, selective medium is Rainbow® agar O157;(28) in particular, it allows the differentiation of *E. coli* O157:H7, other Shiga-like toxin-producing, and non-toxigenic *E. coli*, as black, blue or purple, and red colonies, respectively. Different authors reported comparative evaluation of different chromogenic/fluorogenic media for detecting *E. coli* O157:H7 in food.(38,39) Manafi and Kremsmaier(40) compared Rainbow® agar, Biosynth Culture Medium® O157:H7, Fluorocult HC®, and SMAC obtaining 2.1%, 3.3%, 6.2%, and 7.3%, respectively, of false positive. Validated standard method ISO 16654/2001, used to detect *E. coli* O157 in food, is internationally recognized.(41) This method requires enrichment in mTSB + n for an initial period of 6 h and then for further period of 18 h at 41.5°C, concentration by IMS, and isolation in CT-SMAC. The method is confirmed by biochemical and serological methods, and by characterization of virulence genes *vtx* and *eae*.

Regarding *E. coli* non-O157, there is no standardized enrichment protocol though several studies have been focused on developing a useful method. Different enrichment procedures effective for *E. coli* O157 were found useful for O26; in particular, for isolation from minced beef and radish sprouts the best system was mEC + n at 42°C for 18 h coupled with IMS.(30) The use of novobiocin as a supplement for enrichment media is controversial since novobiocin concentration of 20 mg/l inhibits the growth of some non-O157 and reduces the growth time of Shiga toxin-producing *E. coli*.(42) These authors suggest the careful use of novobiocin in order to minimize false negative results.

The mTSB with cefixime, vancomycin and potassium tellurite is the optimal medium for enrichment of *E. coli* O26, while for O111 the medium is similar without the potassium tellurite.(23) This study highlights that this selective agent should not be added if the aim is recover a wide range of EHEC.

Among different enrichments coupled with IMS, tested also on stressed bacteria, both BPW and BPW + vancomycin have given increased recoveries from minced beef, pasteurized goat's cheese, fresh apple juice, and pepperoni both for *E. coli* O26 and O111 incubated at 42°C rather than at 37°C.(43) These results show the usefulness of IMS and the important role played by the broth chosen, especially considering the physiological state of the bacteria studied. One of the major problems for detecting *E. coli* O26 and O111 by this method remains the lack of suitable solid selective media.(34,43) Although different media have been proposed, such as rhamnose MacConkey medium containing cefixime and tellurite (CT-RMAC) to isolate *E. coli* O26,(23,44,45) problems still remain to detect *E. coli* non-O157 with certainty. The high selectivity of the antibody in the IMS may reduce the problem connected to the other microflora able to grow on the agar medium used.(43) Commercial solid chromogenic media, such as Chromocult and Rainbow® agar, have been suggested as suitable media to isolate *E. coli* O111, O26, O103, and O145.(35)

A chromogenic agar medium – CT-O26 medium – allows the isolation of *E. coli* O26 after enrichment in mEC + n at 42°C for 18 h. This isolation medium was found more effective than the CT-RMAC for ground beef, liver, and alfalfa sprouts and allows the isolation of about 2 cfu of *E. coli* O26 from 25 g food samples after enrichment coupled with IMS.(22) Moreover, the authors highlight the need to improve the usefulness of the medium by a buffer addition that could inhibit hard-too-interpret color changes. To isolate both *E. coli* non-O157 (O26, O103, O111 and O145) and sorbitol-positive and –negative O157 strains, two novel selective media, based on a chromogenic compound to signal *β*-galactosidase activity and fermentative carbon sources, were recently developed.(46)

There is not a validated standard method to detect *E. coli* belonging to O26, O103, O111, and O145 serogroups. The draft of theWorking Group 6 of the Technical Committee 275 of the CEN (TC275/WG6), currently submitted to ISO for evaluation, has been proposed as a method.(25) This method is based on two sequential PCR steps: the first to detect both *vtx* and *eae* virulence genes and the second, in case of positive results, to identify the serogroup associated genes. The method involves (1) enrichment at 37 °C for 18 h in mTSB with 16 mg/l of novobiocin, mTSB with 12 g/l of acriflavin for dairy products, and prewarmed BPW without antibiotics for frozen samples, (2) DNA purification and real-time PCR for *vtx* and *eae* virulence genes, and (3) serogroup specific real-time PCR. Tables 2 and 3 list primers and probes used to detect the virulence genes and to amplify the specific serogroup genes, respectively.

Antibody-based Methods

These methods focus to identify pathogens by using specific antigens and they may be used to confirm the identity of bacteria already isolated or to detect rapidly them prior to isolation. They can use polyclonal or monoclonal antibodies and are classified as homogeneous when the antigen–antibody complex formed is directly measurable – agglutination reactions, immunodiffusion – and heterogeneous when the unbound antibody must be separated from the bound antibody using labeled reagents – sandwich assay (Table 1).

Different type of assays have been developed to give rapid bacteria detection methods(47–49) such as immunofluorescence, immunocapture, enzyme immunoassay, biosensors, and automated immunoassay that are simple to use having all the reagents placed on strips ready-to-use. To obtain an effective method to detect low concentration of *E. coli* O157:H7, the flow cytometry using fluorescein-labeled affinity purified specific antibody together with IMS technique has been developed resulting useful in the detection of as few as 4 *E. coli* cells/g of ground beef in just 7 h of analysis.(50)

The combination of IMS with bioluminescent or fluorescent genetic labeling of *E. coli* O157:H7 has shown good correlation with plate count.(51) In particular, the detection limit of bioluminescent and fluorescent *E. coli* O157:H7 cells was 5×10^3 and 10^6 cfu/ml, respectively. The advantages are reliability, easy procedure and the short time to obtain results (between 1 and 1.25 h).

Wu et al.(48) designed an efficient protocol to detect one *E. coli* O157:H7 cell in 25 g of raw ground beef in 5 h combining, after enrichment, an immunomagnetic bead system with an immunological test. This test gives results in less than 15 min after use of the immunomagnetic system, with detection limits of 0.3 and 0.2 log cfu/25 g, respectively, in mEC + n and in BPW.

The production of polyclonal antibodies specific for Shiga toxin-producing *E. coli* and the development of latex agglutination assays with a specificity approximately of 98% have been recently reported.(52) Other than specificity, advantages are the low cost required for laboratory facilities and for the production of specific polyclonal antiserum.

Another approach for detecting *E. coli* O157:H7 was the combination of flow injection analysis with sandwich immunoassay.(53) This immunosensor based system designed utilize a microcapillary with specific antibodies and immunoliposomes containing sulforhodamine B as the signal amplifiers, so that *E. coli* O157:H7 was detected by measuring the fluorescence intensity. This assay can be performed at room temperature within 45 min, and has a detection limit for heat-killed bacteria of 3.6×10^2 cells/ml.

A monolayer-based piezoelectric immunosensor for *E. coli* O157 detection was proposed by Su and Li.(54) The detection limit for heat-killed pathogen was 10^3 cells/ml and the detection time ranged from 30 to 50 min.

A biosensor array was employed for the detection of *E. coli* O157:H7 in ground beef, turkey sausage, and apple juice taking less than 5 h. The detection limit without sample concentration or enrichment was 5×10^3 and $1–5 \times 10^4$ cells/ml, respectively, in buffer and in the various food matrices.(55) The authors highlight that use of IMS could achieve the detection of one bacterial cell per milliliter.

To detect *E. coli* O157:H7, Song and Vo-Dinh(56) reported the development of a miniaturized biochip system that integrates microsensor detection systems with a microarray of probes used for biochemical assays. In particular, these authors used an array of antibody-immobilized capillary reactors in biochip system performing the enzyme-linked immunosorbent assay (ELISA) and the Cy5 label-based immunoassay. The detection limits of *E. coli* O157:H7 were 3 and 230 cells, respectively, using the ELISA and the Cy5 labelbased immunoassays. Advantages are the low cost of the biochip and its feasibility for multiple immunoassays.

Polymyxin-based ELISA has been developed for detection, after enrichment, of *E. coli* O111 and O26 in ground beef.(57) This system showed sufficient immunological specificity to differentiate the target bacteria from the background food microflora. Advantages are the rapidity, easy procedure and low cost while a disadvantage could be that the high levels of non-target bacteria lead to a reduction of assay signal.

Different authors have pointed out that the use of monoclonal antibodies specific for *E. coli* non-O157(58–61) may allow them to be useful in immunoassay for the rapid detection of these EHEC from food. In fact, monoclonal antibodies, being a reliable source of characterized antibodies, avoid the variability of specificity that characterizes the polyclonal antibodies. In particular, the cross-reactivity among *E. coli* O26 and O111 and other pathogens observed using agglutination with polyclonal antibodies was reduced using monoclonal antibodies specifically developed.(61)

Molecular Methods

Molecular methods offer extremely sensitive and focused techniques to detect and quantify pathogens in food; however, they are more complex and expensive than classic methods. Anyway, molecular methods avoids some problems of classical methods, like variation in enzymatic activity when bacteria are cultured in different media, emergence of biochemical mutants and presence of strain of different species that are very closely related and possess the same phenotype but different genotype.(28) Among these methods, DNA probes, DNA oligonucleotides marked with radioactive isotopes or enzymatic markers that hybridize with single stranded target bacteria DNA, are characterized by rapidity, sensitivity and specificity but have a high cost, are time consuming and require enrichment, and, therefore, are unsuitable for routine detection work. In contrast, PCR techniques, used to produce many copies of a DNA sequence starting from small quantities, are characterized by faster and low cost procedures that guarantee suitable sensitivity. Moreover, using PCR techniques it is possible to increase the DNA copies with limited or no enrichment (Table 1).

Different studies on methods for cellular lysis, DNA release, and their role both on *E. coli* O157:H7 DNA yield and on PCR have been reported.(55) In particular, non-enzymatic methods – heating cell suspensions in distilled water, sodium dodecyl sulfate, PCR buffer, Triton X-100 – and enzymatic methods – lysozyme and proteinase K – have been tested.(62) Maximum yield of DNA was obtained by treating the cells with 2 mg/ml of lysozyme at 37°C for 15 min followed by treatment with 0.8 mg/ml of proteinase K at 55°C for 45 min and then heating at 99.5°C for 10 min. Regarding the effect of lysing methods on PCR, the best conditions were either the treatment of lysed cell with PCR buffer at 99.5°C for 10 min or with proteinase K at 55°C for 15 min followed by heating at 99.5°C for 10 min.

Uyttendaele et al.(63) have evaluated different sample preparation methods on PCR detection of heat-killed *E. coli* O157:H7 in ground beef, highlighting the important role of the DNA extraction method and, in particular, the use of a double washing of the bacterial pellet before the extraction procedure was found useful to eliminate false-positive results.

A simple buoyant density centrifugation technique to prepare PCR samples from food has been proposed to detect *E. coli* O157:H7.(64) This method consists of one minute centrifugation of food homogenate with Percoll® or BacXtractor® gradient medium followed by washing. The detection limits were 1250–2500 cfu/g and 1×10^4 cfu/g for beef and minced beef homogenates, respectively. The brief time of sample preparation allows the processing of many samples and the reduction of time to obtain results.

The efficiency of different DNA extraction methods and the PCR detection limits have been evaluated in the detection of *E. coli* O157:H7 from dairy products.(65) In particular, the yield of DNA recovered using the solvent method was greater than that obtained using the concentration method even if the purity of the DNA obtained from the latter method was better than that obtained from the former. The detection limits ranged from 101 to >106 cfu/ml.

DNA probes and PCR assay have been developed to detect the *stx1* and *stx2* genes,(66,67) the 60 MDa plasmid in *E. coli* O157,(68) the *eae* gene for amplifying a portion common to all EHEC, specifically to O157:H7(69) and to other serotypes,(70) the *uidA* gene,(71) the *fliC* gene,(36) the *rfb* gene,(36,72) and the *gnd* gene.(73)

The use of PCR with SLT1 and SLT2 primers in ground beef has been reported.(74) The detection limits for *E. coli* O157:H7 were 1.2 and 150 cfu/g with and without enrichment, respectively. Different authors have reported the use of multiplex PCR to detect EHEC. In particular, a triplex PCR was developed to detect *E. coli* O26, O111 and O157 in a single reaction. The detection limits in 25 g of sample were ≤10 cfu for *E. coli* O111 and O157 and 15 cfu for *E. coli* O26.(34)

Moreover, the real-time PCR has been used with both the fluorescent intercalating dye (75) and specific probe hybridization. TaqMan probes to detect *E. coli* O26, O55, O91, O103, O111, O113, O145, and O157:H7,(76,77) and Molecular Beacons to detect *E. coli* O157:H7(78) have been used. The PCR-ELISA method to detect the EHEC in milk, cheese, and meat has been used.(79,80) This method is a sensitive tool that allows the rapid screening of food for the presence of EHEC. In a comparative study between the PCR-ELISA and the vero cell assay to detect Shiga toxin-producing *E. coli* in dairy products, Fach et al.(81) found that the former method yielded about 14% more positive results than the latter method.

PCR, real-time PCR or multiplex PCR with the IMS have been used for *E. coli* O157:H7. Gooding and Choudary(82) have developed a method by combining preenrichment, IMS and PCR able to detect *E. coli* O157:H7 in 8 h. Advantages are rapidity, sensitivity, specificity and reproducibility. IMS coupled with real-time PCR gave a specific detection of *E. coli* O157:H7 without culture enrichment.(83) IMS coupled with multiplex PCR allows the use of a single reaction for different serotypes, and has the advantage of concentrating effect.(84)

Recently, the use of loop-mediated amplification assay against the verocytotoxin gene to detect *E. coli* O157 and O26 has been proposed. The authors demonstrate its similarity or superiority to IMS plating method using agar media; so, this assay could be used as an effective way to detect these serotypes.(85)

Commercial Kits and Equipment

Various kits and equipments to detect *E. coli* O157:H7 and *E. coli* non-O157 are produced worldwide.(86–89) Table 4 shows a list of commercial kits available and their current validation status; the most common are described below.

Probelia® (Bio-Rad) allows the screening of food samples for diverse pathogens, among which *E. coli* O157:H7. Based on PCR and sandwich hybridization on microplate, it combines rapidity and accuracy and requires 24 h enrichment in selective media.

TaqMan® *E. coli* O157:H7 Detection Kit (Applied Biosystems, USA) is based on PCR and probes requiring specific instruments such as the Applied Biosystems 7500 real-time PCR System and related materials. It is designed to detect down to 1 cfu in 25 g of food after enrichment.

GeneDisc Cycler® (GeneSystem, Bruz, France) is an automated, miniaturized realtime PCR that uses GeneDisc", a disposable plastic tray preloaded with reagents, to detect the EHEC. In particular, the method screens Shiga toxin-producing *E. coli* according to the virulence genes, followed by identification of the EHEC, according to the specific genes associated to each type.(90) Results obtained with this system(91) have demonstrated its specificity and reliability in the detection of the main EHEC strains; moreover, the GeneSystem allows the simultaneous detection of *E. coli* O157:H7 and *Salmonella* spp. In meat and dairy products.

HQS *E. coli* O157:H7 (ADNucleics, France) allows the detection using Q PCR – plates or strips – and a specific software to interpret the results.

RAPID' *E. coli* O157:H7 (Bio-Rad) foresees enrichment in mTSB with novobiocin for 16–24 h at 41.5°C, IMS step, isolation using a specific agar medium for 24 h at 37°C, and latex test for confirmation of the suspect colonies.

The iQ-Check™ *E. coli* O157:H7 (Bio-Rad) takes advantage of the real-time PCR to obtain results with high sensitivity and specificity in 24 h or less.
Bax® Detection System (DuPont Qualicon, USA) is a fast laboratory method to detect *E. coli* O157:H7 in minced beef, apple cider and orange juice, running up to 96 samples per batch. It consists in the preparation of enriched samples, followed by automated amplification and detection. For minced beef, 14 or 8 h of enrichment is needed using standard media plus novobiocin or Bax® System media, respectively. Bax® *E. coli* O157:H7 MP (DuPont Qualicon, USA) is useful to detect pathogens from raw minced beef and beef trim.
Assurance GDS™ Gene Detection System (BioControl System, Inc., USA) is based on DNA amplification technology and utilizes magnetic particles to capture and transfer *E. coli* O157:H7 from the enriched sample to the amplification tube. Assurance EIA EHEC is based on enzyme immunoassay technology and detects *E. coli* O157 including H7. A multilaboratory collaborative study to detect *E. coli* O157:H7 from orange juice, raw minced beef, and fresh lettuce comparing the GDS™ and standard culture methods concluded that the former method was equivalent to or better than the latter.(92)
Dupont™ Lateral Flow System™ Method (DuPont Qualicon, USA) combines antibodies and colloidal gold on a membrane to detect different pathogens, including *E. coli* O157:H7, from raw beef and processed food. The strip tests can detect 1 cfu in 25 g of sample in 10 min after enrichment ranging from 8 to 18 h using the company recommended media. Advantages are low labor and material cost and minimal training.
Tecra® Visual Immunoassay (TECRA International Pty Ltd, AU) is a fast and specific assay to detect all *E. coli* O157 in food. The kit gives results in 20 h compared with 36 h for standard culture media. Its sensitivity is 1–5 cfu in 25 g of sample and its specificity is > 99%. Other than rapidity, advantages are low cost and easy procedure. Flint and Hartley(93) compared this immunoassay to the standard procedures, concluding that the former is highly sensitive and produces results comparable with those obtained from the latter.
VIDAS *E. coli* O157 ECO (bioMérieux SA) allows detection of the antigen of *E. coli* O157 in food products by an automatic immuno-enzymatic test in 24 h. VIDAS UP *E. coli* O157 including H7 (bioMérieux SA) is an assay, based on the new phage recombinant protein technology, developed for raw beef and veal meats that allows rapid detection with high sensitivity and specificity.
An immunomagnetic separation kit was developed by Denka Seiken Co. (Japan) for detecting *E. coli* O26 and O111, other than O157:H7. Moreover, the same company has developed the *E. coli* O157 Rapid Food Testing based on two enrichment protocols of 6 and 18 h with sensitivity of 1 and 0.04 cfu/g, respectively.
NH Immunochromato O157, O26, and O111 (Cosmo Bio Co., Ltd, Japan) are different kits performed on plate in which anti-*E. coli* O157, O26, and O111 polyclonal antibodies react with the sample after enrichment in mEC broth + n for 18–24 h. The minimum detection sensitivity is between $1 \times 10^4 – 1 \times 10^6$ cfu/ml.
The BioFlash® Biological Identifier is a unit for pathogen detection (Innovative Biosensors, Inc.) based on the Canary® technology, consisting of a cell genetically engineered to recognize a specific pathogen emitting a luminescent signal, developed at the Massachusetts Institute of Technology.(94) It showed sensitivity comparable to that of PCR and allows the detection of < 250 cfu of *E. coli* O157:H7 in different food matrixes requiring only 5 minutes.(95)

Conclusion

Many methods developed and used to detect the EHEC have been described in the literature; however, not all of them are suitable for routine use in the laboratory since they are complex, expensive and require excessive time. Molecular methods are useful, also in supporting the classic methods, to detect rapidly and unequivocally EHEC. One of the problems is the variety of techniques and protocols between laboratories that make inter-laboratory data comparison difficult. Moreover, at the moment, international rules or recommendations are not completely harmonized and this make difficult to monitor and control EHEC. Furthermore, the fact that until recently the incidence of *E. coli* non-O157 in food was considered less common has limited the monitoring and control of foodborne illness caused by these serotypes, and means that only now is a standard method for their detection under consideration. Concerning the availability of various commercial kits, they are not always advantageous over culture methods; indeed, some of them still remain too time-consuming. In conclusion, even if much work and effort have been done in this field, important problem areas remain to address, primarily the development of standard methods for the EHEC non-O157 and of faster detection protocols and the establishment of harmonized worldwide rules.

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References

1. Nataro, J.P.; Kaper, J.B. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **1998**, *11*, 142–201.
2. Caprioli, A.; Morabito, S.; Brugere, H.; Oswald, E. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet. Res. **2005**, *36*, 289–311.
3. McClure, P. The impact of *Escherichia coli* O157 on the food industry. World J. Microb. Biot. **2000**, *16*, 749–755.

4. Morgan, D.; Newman, CP.; Hutchinson, D.N.; Walker, A.M.; Rowe, B.; Majid, F. Verotoxinproducing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiol. Infect. **1993**, *111*, 181–187.
5. Isaäcson, M.; Canter, P.H.; Effler, P.; Arntzen, L.; Bomans, P.; Heenan, R. Haemorrhagic colitis epidemic in Africa. Lancet **1993**, *341*, 961.
6. Weagant, S.D.; Bryant, J.L.; Bark, D.H. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J. Food Protect. **1994**, *57*, 629–631.
7. Centers for Disease Control and Prevention. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami Washington and California. Morb. Mortal. Wkly Rep. **1995**, *44*, 157–160.
8. Deschenes, G.; Casenave, C.; Grimont, F.; Desenclos, J.C.; Benoit, S.; Collin, M.; Baron, S.; Mariani, P.; Grimont, P.A.D.; Nivet, H. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. Pediat. Nephrol. **1996**, *20*, 203–205.
9. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts. Michigan and Virginia. Morb. Mortal. Wkly Rep. **1997**, *46*, 741–744.
10. Hilborn, E.D.;Mermin, J.H.;Mshar, P.A.; Hadler, J.L.; Voetsch, A.;Wojtkunski, C.; Swartz,M.; Mshar, R.; Lambert-Fair, M.A.; Farrar, J.A.; Glynn, M.K.; Slutsker, L. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch. Intern. Med. **1999**, *59*, 1758–1764.
11. Watanabe, Y.; Ozasa, K.; Mermin, J.H.; Griffin, P.M.; Masuda, K.; Imashuku, S.; Sawada, T. Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. Emerg. Infect. Dis. **1999**, *5*, 424–428.
12. Meng, J.; Doyle, M.P.; Zhao, T.; Zhao, S. Enterohemorrhagic *Escherichia coli*. In: *Food Microbiology: Fundamentals and Frontiers*, 2nd ed.; Doyle, M.P.; Beuchat, L.R.; Montville, T.J.; Eds.; ASM Press: Washington, D.C., 2001; 193–213.
13. Murphy, M.; Carroll, A.; Whyte, P.; Mahony, M.; Anderson, W.; McNamara, E.; Fanning, S. Prevalence and characterization of *Escherichia coli* O26 and O111 in retail minced beef in Ireland. Foodborne Pathog. Dis. **2005**, *2*, 357–360.
14. Wilshaw, G.; Thirwell, J.; Jones, A.; Parry, S.; Salmon, R.; Hickey, M. Verocytotoxin producing *Escherichia coli* O157:H7 in beefburgers linked to an outbreak of diarrhea, haemorragic colitis and haemolytic uraemic syndrome in Britain. Lett. Appl. Microbiol. **1994**, *19*, 304–307.
15. ILSI Europe Report Series. Approach to the control of entero-haemorrhagic *Escherichia coli* (EHEC). ILSI Europe Emerging Pathogen Task Force, Brussels, Belgium, 2001.
16. Tuttle, J.; Gomez, T.; Doyle, M.P.; Wells, J.G.; Zhao, T.; Tauxe, R.V.; Griffin, P.M. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol. Infect. **1999**, *22*, 185–192.
17. World Health Organisation (WHO). Report on a WHO working group meeting on shiga-like toxin producing *Escherichia coli* (SLTEC) with emphasis on zoonotic aspects. Bergamo, Italy, Rep. N◦ WHO/CDS/VPH/94.136, 1994.
18. Johnson, R.P.; Clarke, R.C.; Wilson, J.B.; Read, S.C.; Rahn, K.; Renwick, S.A.; Sandhu, K.A.; Alves, D.; Karmali M.A.; Lior, H.; McEwen, S.A.; Spika, J.S.; Gyles, C.L. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenic *Escherichia coli*. J. Food Protect. **1996**, *59*, 1112–1122.
19. Karch, H.; Bielaszewska, M.; Bitzan, M.; Schmidt, H. Epidemiology and diagnosis of shiga toxin-producing *Escherichia coli* infections. Diagn. Micr. Infec. Dis. **1999**, *34*, 229–243.
20. Misselwitz, J.; Karch, H.; Bielazewska, M.; John, U.; Ringelmann, F.; Ronnefarth, G.; Patzer L. Cluster of hemolytic–uremic syndrome caused by toxin-producing *Escherichia coli* O26:H11. Pediatr. Infect. Dis. J. **2003**, *22*, 349– 354.
21. Hussein, H.S.; Sakuma, T. Invited review: prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. J. Dairy Sci. **2005**, *88*, 450–465
22. Hara-Kudo, Y.; Ikedo, M.; Komatsu, O.; Yamamoto, S.; Kumagai, S. Evaluation of a chromogenic agar medium isolation of *Escherichia coli* O26. Food Control. **2002**, *13*, 377–379.
23. Catarame, T.M.G.; O'Hanlon, K.A.; Duffy, G.; Sheridan, J.J.; Blair, I.S.; McDowell, D.A. Optimisation of enrichment and plating procedures for the recovery of *Escherichia coli* O111 and O26 from minced beef. J. Appl. Microbiol. **2003**, *95*, 949–957.
24. Jenkins, C.; Evans, J.; Chart, H.; Willshaw, G.A.; Franke, G. *Escherichia coli* serogroup O26 – a new look at an old adversary. J. Appl. Microbiol. **2008**, *104*, 14–25.
25. Scientific report of EFSA. Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food). The EFSA Journal **2009**, *7*, 1366.
26. Chapman, P.A. Methods available for the detection of *Escherichia coli* O157 in clinical, food and environmental samples. World J. Microb. Biot. **2000**, *16*, 733–740.
27. Hopkins. K.L.; Hilton, A.C. Methods available for the sub-typing of *Escherichia coli* O157. World J. Microb. Biot. **2000**, *16*, 741–748.
28. Beneduce, L.; Spano, G.; Massa, S. *Escherichia coli* O157:H7 general characteristics, isolation and identification techniques. Ann. Microbiol. **2003**, *53*, 511–527.
29. Meng, J.; Zhao, S.; Doyle, D.; Mitchell, S.; Kresovich, S. A multiplex PCR for identifying Shigalike toxin-producing *Escherichia coli* O157:H7. Lett. Appl. Microbiol. **1997**, *24*, 172–176.
30. Hara-Kudo, Y.; Konuma, H.; Nakagawa, H.; Kumagai, S. *Escherichia coli* O26 detection from food using an enrichment procedure and an immunomagnetic separation method. Lett. Appl. Microbiol. **2000**, *30*, 151–154.
31. Šafářiková,M.; Šafářik, I. Immunomagnetic separation of *Escherichia coli* O26, O111 and O157 from vegetables. Lett. Appl. Microbiol. **2001**, *33*, 36–39.

32. Su, X.L.; Li, J. Quantum dot biolabeling couplet with immunomagnetic separation for detection of *Escherichia coli* O157:H7. Anal. Chem. **2005**, *76*, 4806–4810.
33. García-Aljaro, C.; Bonjoch, X.; Blanch, A.R. Combined use of an immunomagnetic separation method and immunoblotting for the enumeration and isolation of *Escherichia coli* O157 in wastewaters. J. Appl. Microbiol. **2005**, *98*, 589–597.
34. Murphy, M.; Carroll, A.; Walsh, C.; Whyte, P.; O'Mahony, M.; Anderson, W.; McNamara, E.; Fanning, S. Development and assessment of a rapid method to detect *Escherichia coli* O26, O111 and O157 in retail minced beef. Int. J. Hyg. Envir. Heal. **2007**, *210*, 155–161.
35. O'Sullivan, J.; Bolton, D.J.; Duffy, G.; Baylis, C.; Tozzoli, R.; Wasteson, Y.; Lofdahl, S. Methods for detection and molecular characterisation of pathogenic *Escherichia coli*. Pathogenic *Escherichia coli* Network, Co-ordination Action FOOD-CT-2006-036256, 2007.
36. Manual of diagnostic tests and vaccines for terrestrial animals. OIE/World Organisation for Animal Health, 2008 (Updated: 14.08.2009) http://www.oie.int/fr/normes/mmanual/2008/pdf/2.09.11_VERO_E_COLI.pdf.
37. Thompson, J.S.; Hodge, D.S.; Borczyk, A.A. Rapid biochemical test to identify vero-cytotoxinpositive strains of *Escherichia coli* serotype O157. J. Clin. Microbiol. **1990**, *28*, 2165–2168.
38. Bettelheim, K.A. Studies of *E. coli* cultured on Rainbow agar O157 with particular reference to enterohaemorrhagic *E. coli* (EHEC). Microbiol. Immunol. **1998**, *42*, 265–269.
39. Restaino, L.; Frampton, E.W.; Turner, K.M.; Allison, D.R. A chromogenic plating medium for isolating *E. coli* O157:H7 from beef. Lett. Appl. Microbiol. **1999**, *29*, 26–30.
40. Manafi, M.; Kremsmaie, B. Comparative evaluation of different chromogenic/fluorogenic media for detecting *Escherichia coli* O157:H7 in food. Int. J. Food Microbiol. **2001**, *71*, 257–262.
41. ISO (International Organization for Standardization). Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157. ISO 16654/2001.
42. Vimont, A.; Delignette-Muller, M.L.; Vernozy-Rozand, C. Supplementation of enrichment broths by novobiocin for detecting Shiga toxin-producing *Escherichia coli* from food: a controversial use. Lett. Appl. Microbiol. **2007**, *44*, 326–331.
43. Drysdale, M.; MacRae, M.; Strachan, N.J.C.; Reid, T.M.S.; Ogden, I.D. The detection of non-O157 *Escherichia coli* in food by immunomagnetic separation. J. Appl. Microbiol. **2004**, *97*, 220–224.
44. Hiramatsu, R.M; Matsumoto, M.;Miwa, Y.; Suzuki, Y.; Saito,M.; Miyazaki, Y. Characterization of Shiga toxin producing *Escherichia coli* O26 strains. J. Clin. Microbiol. **2002**, *40*, 922–925.
45. Murinda, S.E.; Batson, S.D.; Nguyen, L.T.; Gillespie, B.E.; Oliver, S.P. Phenotypic and genetic markers for serotype-specific detection of Shiga-toxin-producing *Escherichia coli* O26 strains from North America. Foodborne Pathog. Dis. **2004**, *1*, 125–135.
46. Possé, B.; De Zutter, L.; Heyndrickx, M.; Herman, L. Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and -negative O157. FEMS Microbiol. Lett. **2008**, *282*, 124–131.
47. de Boer, E.; Beumer, RR. Methodology for detection and typing of foodborne microorganisms. Int. J. Food Microbiol. **1999**, *50*, 119–130.
48. Wu, V.C.H.; Gill, V.; Oberst, R.; Phebus, R.; Fung, D.Y.C. Rapid protocol (5.25 H) for the detection of *Escherichia coli* O157:H7 in raw ground beef by an immuno-capture system (Pathatrix) in combination with colortrix and CT-SMAC. J. Rapid Meth. Aut. Mic. **2004**, *12*, 57–67.
49. Tokarskyy, O.; Marshall, D.L. Immunosensors for rapid detection of *Escherichia coli* O157:H7 – Perspectives for use in the meat processing industry. Food Microbiol. **2008**, *25*, 1–12.
50. Seo, K.H.; Brackett, R.E.; Frank, J.F. Rapid detection of *Escherichia coli* O157:H7 using immunomagnetic flow cytometry in ground beef, apple juice, and milk. Int. J. Microbiol. **1998**, *44*, 115–123.
51. Brovko, L.; Young, D.; Griffiths, M.W. Method for assessment of functional affinity of antibodies for live bacteria. J. Microbiol. Meth. **2004**, *58*, 49– 57.
52. Hajra, T.K.; Bag, P.K.; Das, S.C.; Mukherjee, S.; Khan, A.; Ramamurthy, T. Development of a simple latex agglutination assay for detection of Shiga toxin-producing *Escherichia coli* (STEC) by using polyclonal antibody against STEC. Clinical and Vaccine Immunology **2007**, *14*, 600–604.
53. Ho, J.A.; Hsu, H.; Huang, M. Liposome-based microcapillary immunosensor for detection of *Escherichia coli* O157:H7. Anal. Biochem. **2004**, *330*, 342–349.
54. Su, X.L.; Li, Y. A self-assembled monolayer-based piezoelectric immunosensor for rapid detection of *Escherichia coli* O157:H7. Biosens. Bioelectron. **2004**, *19*, 563–574.
55. Shriver-Lake, L.C.; Turner, S.; Taitt, C.R. Rapid detection of *Escherichia coli* O157:H7 spiked into food matrices. Anal. Chim. Acta **2007**, *584*, 66–71.
56. Song, J.M.; Vo-Dinh, T. Miniature biochip system for detection of *Escherichia coli* O157:H7 based on antibody-immobilized capillary reactors and enzyme-linked immunosorbent assay. Anal. Chim. Acta **2004**, *507*, 115–121.
57. Blais, B.W.; Bosley, J.; Martinez-Perez, A.; Popela, M. Polymixin-based enzyme-linked immunosorbent assay for the detection of *Escherichia coli* O111 and O26. J. Microbiol. Meth. **2006**, *65*, 468–475.
58. Oishi, K.; Koles, N.L.; Guelde, G.; Pollack, M. Antibacterial and protective properties of monoclonal antibodies reactive with *Escherichia coli* O111:B4 lipopolysaccharide: relation to antibody isotype and complementfixing activity. J. Infect. Dis. **1992**, *165*, 34–45.

59. Padhye, N.V.; Doyle, M.P. Production and characterization of a monoclonal antibody specific for enterohemorrhagic *Escherichia coli* of serotypes O157:H7 and O26:H11. J. Clin. Microbiol. **1991**, *29*, 99–103.
60. Kerr, P.; Ball, H.; China, B.; Mainil, J.; Finlay, D.; Pollock, D.; Wilson, I.; Mackie, D. Use of a monoclonal antibody against an *Escherichia coli* O26 surface protein for detection of enteropathogenic and enterohemorrhagic strains. Clin. Diagn. Lab. Immunol. **1999**, *6*, 610–614.
61. Rivera-Betancourt, M.; Keen, J.E.Murine monoclonal antibodies specific for lipopolysaccharide of *Escherichia coli* O26 and O111. Appl. Environ. Microb. **2000**, *66*, 4124–4127.
62. Abolmaaty, A.; El-Shemy, M.G.; Khallaf, M.F.; Levin, R.E. Effect of lysing methods and their variables on the yield of *Escherichia coli* O157:H7 DNA and its PCR amplification. J. Microbiol. Meth. **1998**, *34*, 133–141.
63. Uyttendaele, M.; van Boxstael, S.; Debevere, J. PCR assay for detection of the *Escherichia coli* O157:H7 *eae*-gene and effect of the sample preparation method on PCR detection of heat-killed *Escherichia coli* O157:H7 in ground beef. Int. J. Food Microbiol. **1999**, *2*, 85–95.
64. Lindqvist, R. Preparation of PCR samples from food by a rapid and simple centrifugation technique evaluated by detection of *Escherichia coli* O157:H7. Int. J. Food Microbiol. **1997**, *37*, 73–82.
65. McKillip, J.L.; Jaykus, L.A.; Drake, M.A. A comparison of methods for the detection of *Escherichia coli* O157:H7 from artificially-contaminated dairy products using PCR. J. Appl. Microbiol. **2000**, *89*, 49–55.
66. Karch, H.; Meyer, T. Evaluation of oligonucleotide probes for identification of Shiga-like-toxin-producing *Escherichia coli*. J. Clin. Microbiol. **1989**, *27*, 1180–1186.
67. Paton, J.C.; Paton, A.W. Pathogenesis and diagnosis of a Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **1998**, *11*, 450–479.
68. Levine,M.; Xu, J.; Kaper, J.; Lior, H.; Prado, V.; Tall, B.; Cataro, J.; Karch, H.;Wachsmuth, K. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and haemolytic uremic syndrome. J. Infect. Dis. **1987**, *156*, 175–182.
69. Gannon, V.P.J.; Rashed, M.; King, R.K.; Golsteyn-Thomas, E.J. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. J. Clin. Microbiol. **1993**, *31*, 1268–1274.
70. Louie, M.; De Azavedo, J.; Clarke, R.; Borczyk, A.; Lior, H.; Richter, M.; Brunton, J. Sequence heterogeneity of the eae gene and detection of verocytotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol. Infect. **1994**, *112*, 449–461.
71. Feng, P. Identification of *Escherichia coli* serotype O157:H7 by DNA probe specific for an allele of *uidA* gene. Mol. Cell. Probe **1993**, *7*, 151–154.
72. Desmarchelier, P.M.; Bilge, S.S.; Fegan, N.; Mills, L.; Vary, J.C.; Tarr, P.I. A PCR specific for *Escherichia coli* O157 based on the *rfb* locus encoding O157 lypopolysaccharide. J. Clin. Microbiol. **1998**, *36*, 1801–1804.
73. Gilmour, M.W.; Olson, A.B.; Andrysiak, A.K.; Ng, L.; Chui, L. Sequence-based typing of genetic targets encoded outside of the O-antigen gene cluster is indicative of Shiga toxin-producing *Escherichia coli* serogroup lineages. J. Med. Microbiol. **2007**, *56*, 620–628.
74. Guan, J.; Levin, R.E. Quantitative detection of *Escherichia coli* O157:H7 in ground beef by the polymerase chain reaction. Food Microbiol. **2002**, *19*, 159–165.
75. Yoshitomi, K.J.; Jinneman, K.C.; Weagant, S.D. Detection of Shiga toxin genes *stx1*, *stx2*, and the +93 *uidA* mutation of *Escherichia coli* O157:H7/H-using SYBR® Green I in a real-time multiplex PCR. Mol. Cell. Probe. **2006**, *20*, 31–41.
76. Perelle, S.; Dilasser, F.; Grout, J.; Fach, P. Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. Mol. Cell. Probe. **2004**, *18*, 185–192.
77. Perelle, S.; Dilasser, F.; Grout, J.; Fach, P. Detection of *Escherichia coli* serogroup O103 by realtime polymerase chain reaction. J. Appl. Microbiol. **2005**, *98*, 1162–1168.
78. Fortin, N.Y.; Mulchandani, A.; Chen, W. Use of real-time polymerase chain reaction and molecular beacons for detection of *Escherichia coli* O157:H7. Anal. Biochem. **2001**, *289*, 281–288.
79. Cocolin, L.; Astori, G.; Manzano, M.; Cantoni, C.; Comi, G. Development and evaluation of a PCR-microplate capture hybridization method for direct detection of verotoxigenic *Escherichia coli* strains in artificially contaminated food samples. Int. J. Food Microbiol. **2000**, *54*, 1–8.
80. Perelle, S.; Dilasser, F.; Grout, J.; Fach, P. Screening food raw materials for the presence of the world's most frequent clinical cases of Shiga toxin-encoding *Escherichia coli* O26, O103, O111, O145 and O157. Int. J. Food Microbiol. **2007**, *113*, 284–288.
81. Fach, P.; Perelle, S.; Dilasser, F.; Grout, J. Comparison between a PCR-ELISA test and the vero cell assay for detecting Shiga toxin-producing *Escherichia coli* in dairy products and characterization of virulence traits of the isolated strains. J. Appl.Microbiol. **2001**, *90*, 809–818.
82. Gooding, C.M.; Choudary, P.V. Detection of *Escherichia coli* O157:H7 in ground beef in eight hours. J. Microbiol. Meth. **1998**, *34*, 89–98.
83. Fu, Z.; Rogelj, S.; Kieft, T.L. Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real-time PCR. Int. J. Food Microbiol. **2005**, *99*, 47–57.
84. Sarimehmetoglu, B.; Aksoy, M.H.; Ayaz, N.D.; Ayaz, Y.; Kuplulu, O.; Kaplan, Y.Z. Detection of *Escherichia coli* O157:H7 in ground beef using immunomagnetic separation and multiplex PCR. Food Control **2009**, *20*, 357–361.

85. Hara-Kudo, Y.; Konishi, N.; Ohtsuka, K.; Hiramatsu, R.; Tanaka, H.; Konuma, H.; Takatori, K. Detection of Verotoxigenic *Escherichia coli* O157 and O26 in food by plating methods and LAMP method: A collaborative study. Int. J. Food Microbiol. **2008**, *122*, 156–161.
86. Cosmo Bio Co., Ltd. Ispiration for Life Science. http://www.cosmobio.co.jp/export_e/products/bio_medical/products_nph_20090806.asp?entry_id=3871.
87. Denka Seiken Co., Ltd. Manufacturer of diagnostic reagents and vaccines. http://denkaseiken.jp/english/products/bacteriology/escherichiaColi.html
88. MATRIX MicroScience, http://www.matrixmsci.com/pathatrix.htm#results
89. TECRA International Pty Ltd, http://www.tecra.net/products/e._coli_o157
90. Hallier-Soulier, S.; Fach, P.; Beutin, L. Ready-to-use multiplex real time PCR assays for the detection of STEC & the identification of EHEC. http://www.genesystems.fr/images/stories/food/STEC/poster%20iafp-ready-to-use%20multiplex%20real%20time%20pcr%20assays.pdf
91. Beutin, L.; Jahn, S.; Fach, P. Evaluation of the GeneDisc real-time PCR system for detection of enterohaemorrhagic *Escherichia coli* (EHEC) O26, O103, O111, O145 and O157 strains according to their virulence markers and their O- and H-antigen-associated genes. J. Appl. Microbiol. **2009**, *106*, 1122–1132.
92. Feldsine, P.T.; Green, S.T.; Lienau, A.H.; Stephens, J.; Jucker, M.T.; Kerr, D.E. Evaluation of the Assurance GDS^{TM} for *Escherichia coli* O157:H7 Method and Assurance GDS for Shiga toxin Genes Method in Selected Foods: Collaborative Study. J. AOAC Int. **2005**, *88*, 1334–1348.
93. Flint, S.H.; Hartley, N.J. Evaluation of the TECRA *Escherichia coli* 0157 visual immunoassay for tests on dairy products. Lett. Appl. Microbiol. **1995**, *21*, 79–82.
94. Rider, T.H.; Petrovick, M.S.; Nargi, F.E.; Harper, J.D.; Schwoebel, E.D.; Mathews, R.H.; Blanchard, D.J.; Bortolin, L.T.; Young, A.M.; Chen, J.; Hollis, M.A. A B cell-based sensor for rapid identification of pathogens. Science **2003**, *301*, 213–215.
95. Ellingson, J.L.; Koziczkowski, J.J.; Anderson, J.L.; Carlson, S.A.; Sharma, V.K. Rapid PCR detection of enterohaemorrhagic *Escherichia coli* (EHEC) in bovine food products and feces. Mol. Cell. Probe. **2005**, *19*, 213–217.
96. Nielsen, E.M.; Andersen, M.T. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5' nuclease PCR assay. J. Clin. Microbiol. **2003**, *41*, 2884–2893.

Table 1. Methods currently used to detect enterohaemorrhagic *Escherichia coli* strains, reported according to technology used, target, microbial serogroup, substrate on which they were tested, detection limit, and time of analysis

Type of method	Microbial serogroup	Food or substrate spiked to test the method	Target of the method	Detection limit	Time of analysis	Ref.
PCR						
PCR	O157:H7	Ground beef	*eae* gene	10^5 cfu/g	<14 h	63
PCR	O157:H7	Ground beef	SLT1 and SLT2 genes	150 cfu/g (without enrichment) 1.2 cfu/g (with enrichment)	60 min (without enrichment) 5–6 h (with enrichment)	74
PCR preceded by two different DNA extractions	O157:H7	Brie cheese, Cheddar cheese, whey powder	*slt-II* gene	Brie cheese: 10^1 cfu/ml – solvent method, 10^2 cfu/ml – concentration method;Cheddar cheese: 10^1 cfu/ml – both methods whey powder: $\geq 10^6$ cfu/ml – solvent method, 10^4 cfu/ml – concentration method	< 5 h	65
Triplex-PCR preceded by enrichment in mTSB	O26, O111, and O157	Minced beef	*rfb* and *wzx* genes	O26: 15 cfu/25g O111: 10 cfu/25g O157: <10 cfu/25g	24 h	34
Real-time PCR and molecular Beacons using InstaGene matrix kit	O157:H7	Raw milk, apple juice	*rfbE* gene	2×10^2 cfu/ml	3 h	78
PCR-ELISA preceded by enrichment in modified EC	EHEC	Dairy products	*stx1* and *stx2* genes	10^2 cfu/ml	24 h	81
Real-time PCR prededed by IMS enrichment	O157:H7	Minced beef	Immunomagnetic beads *eaeA* gene	1.3×10^4 cells/g	6–8 h	83
PLATING						
Plating	O157:H7	Raw minced beef	BCM/MSA-BCIG	0.29–3.12 cfu/g	48 h	39
Plating preceded by IMS enrichment	O26	Raw ground beef, liver, fresh alfalfa sprouts, lettuce	mEC + n Immunomagnetic beads CT-O26 medium	2 cfu/25 g	42 h	22
Plating preceded by IMS enrichment	O26	Various food (see "detection limit" column)	Immunomagnetic beads BPW, BPW-V	Using BPW: minced beef 126, cheese 132, apple juice 157, and pepperoni 17 cfu/g;Using BPW-V: minced beef 114, cheese 69, apple juice 127, and pepperoni 19 cfu/g	24 h	43
Plating preceded by IMS enrichment	O111	Various food (see "detection limit" column)	Immunomagnetic beads BPW, BPW-V	Using BPW: minced beef 138, cheese 62, apple juice 170, and pepperoni 248 cfu/g Using BPW-V: minced beef 184, cheese 65, apple juice 188, and pepperoni 197 cfu/g	24 h	43
ANTIBODIES						
Polymyxin-ELISA preceded by enrichment in mTSBn	O26 and O111	Ground beef	LPS antigens	0.4 cfu/g (O26);0.25 cfu/g (O111)	48 h	57

Table 1. (continued)

Type of method	Microbial serogroup	Food or substrate spiked to test the method	Target of the method	Detection limit	Time of analysis	Ref.
Polymyxin-ELISA preceded by enrichment in O111SB	O111	Ground beef	LPS antigens	0.19 cfu/g	48 h	57
Polymyxin-ELISA preceded by enrichment in O26SB	O111	Ground beef	LPS antigens	0.44 cfu/g	48 h	57
Flow cytometry and immunomagnetic bead (IMFC)	O157:H7	Ground beef	*E. coli* O157:H7 antibodies	4 cells/g	7 h	50
Array biosensor	O157:H7	Ground beef, turkey sausages, apple juice	*E. coli* O157:H7 antibodies	1–5 × 10^4 cells/ml	<5 h	55
RapidCheck® Lateral Flow Test Kit	O157	Raw minced beef	*E. coli* O157:H7 antibodies	1 cfu/25g	<18 h	49
Singlepath® *E. coli* O157 rapid Lateral Flow Assay	O157	Raw minced beef, pasteurized milk	*E. coli* O157:H7 antibodies	10^4–10^6 cfu/ml	18–24 h	49
Pathatrix + Colotrix	O157:H7	Raw ground beef	Immunomagnetic beads, antibodies	0.3–0.2 cfu/25g	5 h	48
NH Immunochromato O157, O26, and O111	O157, O26, and O111	Various food	*E. coli* O-specific antibodies	1 × 10^4–1 × 10^6 cfu/ml	18–24	86
E. coli O157 Rapid Food Testing	O157	Various food	Magnetic beads with antibodies	1 cfu/g (6h enrichment)0.04 cfu/g (18h enrichment)	<18 h	87
Pathatrix ULTRA/Auto *E.coli* O157 Test System	O157	Various food	Re-circulatingIMS	1–10 cfu/25g	5–6 h	88
TECRA *E. coli* O157 Visual Immunoassay and TECRA *E. coli* O157 Immunocapture	O157	Meat products, milk and dairy products, vegetables, salads and fruit juices	O-specific antibodies	1–5 cfu/25g	20 h	89

Table 2. Primers and probes used in Polymerase Chain Reaction assay to detect virulence genes of *E. coli* non-O157 according to the draft of the European Committee for Standardization TC275/WG6. Source: taken in part from.(25)

Target gene (Ref.)	Forward primer, reverse primer and probe sequences (5'–3')[a]	Amplicon size (bp)	Location within sequences
vtx1[76]	TTTGTYACTGTSACAGCWGAAGCYTTACG	131	878–906
	CCCCAGTTCARWGTRAGRTCMACRTC		983–1008
	Probe-CTGGATGATCTCAGTGGGCGTTC TTATGTAA		941–971
vtx2[76]	TTTGTYACTGTSACAGCWGAAGCYTTACG	128	785–813
	CCCCAGTTCARWGTRAGRTCMACRTC		785–813
	Probe-TCGTCAGGCACTGTCTG AAACTGCTCC		838–864
eae[96]	CAT TGA TCA GGA TTT TTC TGG TGA TA	102	899–924
	CTC ATG CGG AAA TAG CCG TTA		1000–979
	Probe-ATAGTCTCGCCAGTATTCG CCACCAATACC		966–936

[a]In the sequence Y is (C, T), S is (C, G), W is (A, T), R is (A, G), M is (A, C).

Table 3. Primers and probes used to amplificate the *E. coli* non-O157 O-antigen-specific genes according to the draft of the European Committee for Standardization TC275/WG6. Source: taken in part from.(25)

Target gene (serogroup)	Forward primer, reverse primer and probe sequences (5'–3')	Amplicon size (bp) (Ref.)	Location within sequences
rfbE (O157)	TTTCACACTTATTGGATGGTCTCAA	88[76]	348–372
	CGATGAGTTTATCTGCAAGGTGAT		412–435
	Probe-AGGACCGCAGAGGAAAGAGAG GAATTAAGG		381–410
wbdI (O111)	CGAGGCAACACATTATATAGTGCTTT	146[76]	3464–3489
	TTTTTGAATAGTTATGAACAT CTTGTTTAGC		3579–3609
	Probe-TTGAATCTCCCAGATGATCA ACATCGTGAA		3519–3548
wzx (O26)	CGCGACGGCAGAGAAAATT	135[76]	5648–5666
	AGCAGGCTTTTATATTCTCCAACTTT		5757–5782
	Probe-CCCCGTTAAATCAATACTATT TCACGAGGTTGA		5692–5724
ihp1 (O145)	CGATAATATTTACCCCACCAGTACAG	132[76]	1383–1484
	GCCGCCGCAATGCTT		1500–1514
	Probe-CCGCCATTCAGAATGC ACACAATATCG		1472–1498
wzx (O103)	CAAGGTGATTACGAAAATGCATGT	99[77]	4299–4323
	GAAAAAAGCACCCCCGTACTTAT		4397–4375
	Probe-CATAGCCTGTTGTTTTAT		4356–4373

Table 4. Mainly available commercial kits to detect enterohaemorrhagic *Escherichia coli* strains and their current validation status

Trade name	Manufacturer	Validation
HQS *E.coli* O157:H7	ADNucleis	AFNOR
VIDAS UP *E.coli* O157 including H7	bioMérieux SA	AFNOR, AOAC in progress
VIDAS *E. coli* O157 (ECO)	bioMérieux SA	AFNOR, AOAC, chinese government
RAPID'*E.coli* O157:H7	Bio-Rad	AFNOR, AOAC
iQ-Check *E.coli* O157:H7	Bio-Rad	AFNOR, AOAC
BAX® *E. coli* O157:H7 MP	DuPont Qualicon	AFNOR, AOAC
GeneDisc" *E. coli* O157:H7	GeneSystems	AFNOR
TECRA *E. coli* O157 Visual Immunoassay and TECRA *E. coli* O157 Immunocapture	TECRA	AOAC, Health Canada, Ministry of Agriculture of Brazil, Meat Industry Research Institute New Zealand
Pathatrix ULTRA/Auto *E.coli* O157 Test System	Matrix MicroScience Ltd	AOAC
Assurance GDS™ Gene Detection System	BioControl System, Inc.	AOAC
Singlepath® *E. coli* O157 Lateral Flow Assay	Merck KGaA / EMD Chemicals, Inc.	AOAC
ADIAFOOD Rapid Pathogen Detection System for *E. coli* O157 also H7	AES Chemunex	AOAC
Duopath® Verotoxin Lateral Flow Assay	EMD Chemicals, Inc.	AOAC
BBL™ CHROMagar™ O157	BD Diagnostics	AOAC
DuPont Lateral Flow System *E. coli* O157 Test Kit	DuPont Qualicon	AOAC
TEMPO® EC Test	bioMérieux SA	AOAC
LightCycler® foodproof *E. coli* O157 Detection Kit with ShortPrep foodproof II Kit	Roche Diagnostics GmbH	AOAC
RapidChek® SELECT™ *E. coli* O157	Strategic Diagnostics, Inc.	AOAC
FoodChek™ *E.coli* O157	FoodChek Systems, Inc.	AOAC
E.coli O157:H7 LT Test Kit	DuPont Qualicon	AOAC
NH Immunochromato O157, O26, and O111	Cosmo Bio Co., Ltd	not referred
BioFlash® Biological Identifier	Innovative Biosensors, Inc.	not referred
TaqMan® *E. coli* O157:H7 Detection Kit	Applied Biosystems	not referred
Probelia®	Bio-Rad	not referred
E. coli O157 Rapid Food Testing	Denka Seiken Co.	not referred