

Chapter 2

Isolation and Detection of Pathogenic *Escherichia coli* in Foods

2.1 Introduction

Microbiological analysis and detection of a target microorganism(s) within a food can involve various steps and may include visual, biochemical, immunological, or genetic methods either before enrichment (quantitative or enumerative methods) or after enrichment (qualitative methods or presence/absence tests) (Lopez-Campos et al. 2012). Conventional or traditional methods for detecting microorganisms in foods can often involve enriching in one or more liquid enrichment media that allow for the resuscitation and multiplication of a particular microorganism. Subsequently, the isolation of the target microorganisms can occur when the enrichment is grown on selective and/or differential plating media for visual and additional confirmation steps (Jasson et al. 2010). Many standardized methods for selected food-borne pathogens are available and are considered the reference analytical methods for official controls and often involve conventional methods (Jasson et al. 2010). Although these methods are rather sensitive for the detection of pathogens, they are laborious and time-consuming, often requiring several days before results are known (Lopez-Campos et al. 2012). Therefore, recent research efforts have concentrated on developing methods that can reduce the assay time through the use of alternatives or combinations of isolation and detection methods and even automated versions wherever possible (Jasson et al. 2010; Lopez-Campos et al. 2012).

Pathogenic *E. coli* represents a phenotypically diverse group of pathogens and there is currently no single method that can be used to enrich, isolate, or select for the various pathotypes that exist. Consequently, available methods have been developed to detect or isolate particular pathotypes of interest. Developments of microbiological analysis of pathogenic *E. coli* in food has predominately focused on the Shiga-toxin producing *E. coli* (STEC) group, particularly the serogroup O157 and recently, other serogroups of concern. As a result, a number of methods have been published but various challenges in isolating these pathogens are currently not resolved. Often fecal samples from ill patients will contain large numbers of the pathogen that aid in detection and isolation, but for foods the predominant challenge is that these pathogens are usually present in very low numbers, in a

non-homogenous distribution among very high levels of background microflora in complex matrices. Various inhibitors within the food matrices can also interfere with isolation and subsequent detection methods. In addition, these STEC cells may be present in an injured or stressed state as a result of unfavorable conditions during food processing, such as exposure to different pH, temperatures, and the presence of preservatives (Grant et al. 2011; Wang et al. 2013).

The complexity of food matrices is the major obstacle for the development of effective sampling and rapid testing methods. Enrichment is primarily used to resuscitate injured/stressed target cells, increase the target cell numbers, as well as dilute the effects of food inhibitors and background flora within the assay (Wang et al. 2013). However, the time taken to enrich samples can lengthen the isolation process to days as opposed to hours (Ge and Meng 2009; Wang et al. 2013). In addition, these problems result in a necessary trade-off between the need to incorporate selective agents such as antibiotics and inhibitory agents to favor the growth of the STEC while suppressing the unwanted background flora without potentially inhibiting the stressed and injured cells by these agents (O'Sullivan et al. 2007). Effective sampling and sample preparation prior to the actual analyses is, therefore, a critical step in the isolation process (Wang et al. 2013).

2.2 General Method of Isolation for *E. coli*

The isolation and identification of the *E. coli* pathotypes that are not STEC is difficult due to the lack of a medium that can be used to enrich or select for specific strains. An isolation method for all pathogenic *E. coli* is outlined in the Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) (Feng et al. 2011a). The FDA-BAM method is a general procedure for the isolation of *E. coli* (excluding STEC) before subsequent testing for specific virulence traits of different pathotypes. In brief, the method recommends pre-enrichment of a 25 g food sample in 225 ml brain heart infusion (BHI) broth at 35 °C for 3 h to facilitate resuscitation of sublethally injured cells. The pre-enrichment is then transferred to 225 ml of tryptone phosphate (TP) broth and incubated at 44 °C for 20 h. A volume of enriched broth is then plated onto Levine's eosin-methylene blue (L-EMB; colonies produce a green metallic sheen) agar and MacConkey agar plates (colonies are brick red in color). These plates should be incubated at 37 °C for 24 h. Colony morphology and color may vary among pathogenic *E. coli* strains and Enteroinvasive *E. coli* (EIEC) do not ferment lactose; therefore, it is recommended that at least 10 typical and 10 atypical colonies should be picked for further analysis. It is important to note that this method is useful for isolating Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), and Enterotoxigenic *E. coli* (EAEC), but not STEC O157:H7, which does not grow well at 44 °C. Specific characteristics and methods to identify and confirm presumptive *E. coli* are described for each pathotype below.

2.3 Shiga Toxin-Producing *E. coli*

2.3.1 Culture and Isolation of Shiga Toxin-Producing *E. coli*

E. coli O157:H7 is phenotypically distinct from other *E. coli* as it can exhibit a delayed (negative) fermentation of D-sorbitol and does not demonstrate glucuronidase activity, hence these traits are often used to selectively isolate *E. coli* O157:H7 from foods (Thompson et al. 1990; Feng et al. 2011a). Unlike *E. coli* O157:H7, there is currently no standardized method for the isolation of non-O157 STEC from foods. The primary limitation for the detection of non-O157 STEC is the lack of known physiological characteristics that distinguish more than 400 serogroups of non-O157 STEC from non-pathogenic or commensal *E. coli*, which hampers the effective detection and enumeration of these organisms (Coombes et al. 2008; Mathusa et al. 2010; Mingle et al. 2012). In addition, to date, it is still not possible to fully define human pathogenic STEC strains, which adds to the difficulty of identifying clinically significant strains in humans (EFSA 2013).

Existing methodology for STEC is based on developments relating to *E. coli* O157:H7, and generally two approaches have been used to detect the pathogens in foods. Firstly, a primary enrichment to recover *E. coli*, including STEC, is used and subsequently screened for selected virulence factors and the subsequent isolation of an STEC is attempted using available methods (O'Sullivan et al. 2007). This can often result in the isolation of non-disease causing strains. The second approach involves serotype-dependent methods that target specific serogroups frequently associated with human disease and outbreaks such as strains belonging to the serogroups O26, O45, O91, O103, O111, O121, O145, and O157 (Farrokh et al. 2013). The disadvantage of this approach is that serogroups that are less frequently associated with disease or are newly emerging (for example, the *E. coli* O104:H4 serotype associated the large European outbreak; Inset 1.2) are missed in these analyses.

The two most common and successful enrichment media used for *E. coli* O157:H7 and other STEC serotypes are tryptone soy broth (TSB) and *E. coli* broth (EC) with or without modifications to their original formulation (O'Sullivan et al. 2007). These modifications include bile salts or dipotassium phosphate in TSB (mTSB) or less bile salts in modified EC broth, as well as various other selective components which aid in the pathogens' recovery. The International Organization for Standardization (ISO) method 16654 for foods and animal stuffs recommends enrichment in mTSB with novobiocin (mTSBn) at 41.5°C for an initial period of 18–24 h with subsequent analysis at 6 and 24 h.

The FDA-BAM method (Feng et al. 2011a) recommends the use of buffered peptone water with pyruvate (mBPWp) which contains several antimicrobial reagents that effectively suppress normal flora growth and non-target competitors, yet allows the growth of viable *E. coli* O157:H7 and other STEC and is capable of detecting <1 cfu/g in foods. This method also describes a screening step of food enrichments using a real-time PCR (RT-PCR) protocol to rapidly rule out negative samples or

establish the presumptive presence of *E. coli* O157:H7 in a sample (see Sect. 2.3.2). The enrichment procedure and RT-PCR screening assay has also been validated for the detection and recovery of other non-O157 STEC but is not serogroup specific.

The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) has released a laboratory guidebook for the detection and isolation of STEC from meat products, carcasses, and environmental sponges (MLG 5B.05) (USDA-FSIS 2013). Target STEC strains are often those that have been noted as adulterants of raw non-intact beef products and product components in the USDA-FSIS register (USDA-FSIS 2013). These include STEC belonging to the O157 serogroup or any one of the following six non-O157 serogroups; O26, O45, O103, O111, O121, and O145. Shiga-toxin producing *E. coli* belonging to these six serogroups are often collectively referred to as the “Big 6 STEC” or “Top 7 STEC” when this group includes *E. coli* O157:H7 (Wang et al. 2013; USDA-FSIS 2014b). While the USDA-FSIS protocol (MLG 5B.05) is designed to guide FSIS laboratories required to perform regulatory testing of meat products, many establishments have chosen to implement STEC screen tests using a method based on, or demonstrated to be equivalent to, the FSIS guidebook.

The USDA-FSIS method (MLG 5B.05) recommends the use of mTSB enrichment without any antibiotics such as sodium novobiocin which is commonly used for *E. coli* O157:H7. Exclusion of sodium novobiocin allows the analysis of samples such as raw beef product, environmental and carcass sponges for *E. coli* O157:H7, and the other STEC noted as adulterants in the USDA-FSIS register (USDA-FSIS 2013). Following a screening step of the food enrichment using RT-PCR (refer to Sect. 2.3.2), the positive enrichments are processed using an immunomagnetic separation (IMS) method which is a technique that is also recommended in other methods (Inset 2.1).

Inset 2.1: Immunomagnetic Separation (IMS)

Many pathogens are usually present in low cell numbers within a food sample and the use of immunomagnetic separation (IMS) has provided an enhanced isolation capacity by aiding in the capture, separation, and concentration of a target pathogen from a sample matrix (Stevens and Jaykus 2004; Grant et al. 2011). The method uses superparamagnetic (i.e., only exhibit magnetic properties in the presence of an external magnetic field) or polystyrene beads that are coated with specific antibodies that capture the intact pathogen present within a complex suspension such as an enrichment broth (Deisingh and Thompson 2004). The application of a magnetic field attracts the beads along with the attached intact and viable pathogen and allows for the cell-bead complex to be extracted and concentrated in a tube. Any non-specific organic or liquid material carried over on the beads is removed through wash steps and the beads are subsequently released and plated onto selective media for further isolation. The method does not yield a pure culture and therefore requires the use of other methods to aid identification of the target bacteria, either

through conventional or molecular assays (Olsvik et al. 1994). Indeed IMS has the advantage of being very versatile and can be used in combination with different rapid and automated assays for the detection of *E. coli* O157:H7 (Fu et al. 2005; Hunter et al. 2011).

Over the years, IMS has seen continual developments for the isolation of *E. coli* O157:H7 from foods and is included as a step in the gold standard cultural method for the pathogen (ISO 16654) and is now also commercially available for other key serotypes (e.g., O26, O103, O111, O121, O145). The utilization of IMS for key non-O157 serotypes is becoming more common and studies have reported the usefulness of serotype-specific enrichment broth, IMS, and selective agar with serological and biochemical confirmation testing for the isolation of these serotypes (Catarama et al. 2003; Bettelheim 2007). Immunomagnetic separation in combination with selective enrichment has been found to improve rates of detection and isolation of *E. coli* O157:H7 in complex food matrices (Onoue et al. 1999; Weagant and Bound 2001). However, it has been reported that the detection of some serotypes (O26 and O111) through IMS was affected by enrichment protocol, high numbers of background microflora, and the physiological state of the organism. It was suggested that recovery may be improved by using media with low nutrients, such as buffered peptone water instead of tryptic soy broth and using higher enrichment temperatures (Drysdale et al. 2004).

The most frequently used magnetic carriers are Dynabeads (produced by Dynal, Oslo, Norway), which are polystyrene-based particles ranging from 2.8–4.5 μM . There are also commercially available automated IMS platforms and include the BeadRetriever™ (Dynal Biotech Ltd, Wirral, UK) and PATHATRIX® (Applied Biosystems, Foster City, CA, USA). The later version has been AOAC Research Institute approved for the detection/isolation of *E. coli* O157:H7 and uses up to 250 ml of recirculating enrichment broth over the trapped beads in order to increase the sensitivities and reduce detection times (Fedio et al. 2011). Other systems such as the Assurance GDS® systems (BioControl, USA) are designed specifically to aid in the confirmation of *E. coli* O157:H7 and the additional “Big 6 STEC” serogroups. The system involves the use of a kit that contains IMS particles and components to be used for a RT-PCR assay that is performed on a specific GDS machine. The kit contains Poly-IMS—Top STEC beads which contain a mixture of IMS particles targeted against the Top 7 STEC. The Assurance GDS PickPen® collects the IMS particles that have captured the Top 7 serogroups from an aliquot of a positive enrichment. The RT-PCR assay can detect *E. coli* O157:H7 or the remaining “Big 6 STEC” as well as the *stx*₁, *stx*₂, and *eae* genes to provide an overall confirmation of a positive “Top 7.” Following a positive PCR reaction, the IMS procedure is repeated again, either with the Poly-IMS—Top STEC or individual IMS beads (IMS Panel-Top STEC) which allows the capture and isolation of each of the specific 7 target O-groups prior to selective agar plating and confirmation.

The optimization of selective enrichment of non-O157 STEC is ongoing. A review of various enrichment protocols assessed key variables, including type of broth medium, presence of antibiotics and or selective ingredients, and incubation time/temperature conditions, however, no clear conclusions could be drawn from the study. It was highlighted that more extensive work would be required with multiple serotypes and sample matrices (Vimont et al. 2006; Grant et al. 2011) and that all serotypes cannot be detected by one method (Baylis 2008).

The most widely used solid plating medium for the detection of non-sorbitol fermenting *E. coli* O157:H7 is sorbitol MacConkey (SMAC) agar, and selectivity is also improved by the addition of selective supplements cefixime and potassium tellurite (CT-SMAC) (Zadik et al. 1993). However, this media is not appropriate for the detection of non-O157 and sorbitol-fermenting *E. coli* O157:H7 which have also been implicated in human disease (Mathusa et al. 2010). Diagnosis of sorbitol-fermenting non-O157 STEC is complex and requires non-culture screening strategies because selective and differential media are not available for their culture (Gould et al. 2009). This is seldom applied, even in specialized laboratories, resulting in under diagnosis of this pathogen (Werber et al. 2011).

A variety of chromogenic media have become available commercially in recent years for the detection of *E. coli* O157:H7 in humans, food, and animal feed stuffs. These media contain a particular mixture of artificial chromogenic conjugates composed of a substrate for an *E. coli*-specific enzyme coupled to a chromophore. When the *E. coli* enzyme cleaves the colorless conjugate, one or more insoluble chromophores are released, resulting in a distinctive color for the *E. coli* colonies (Gouali et al. 2013). Current chromogenic agars use the characteristic traits such as sorbitol fermentation, glucuronidase or galactosidase activity and are largely effective for the discrimination of *E. coli* O157:H7. Chromogenic agars that are specific for the isolation of *E. coli* O157:H7 from foods include CHROMagar™ O157 (CHROMagar Microbiology, Paris, France), RAPID'E. coli™ O157:H7 (Biorad, Hercules, CA, USA), and Rainbow® O157 agar (Biolog, Hayward, CA, USA). Studies have been conducted to determine whether chromogenic culture media developed for the detection of *E. coli* O157:H7 are also applicable to non-O157 serotypes; however the variability of phenotypic characteristics for non-O157 on these media impedes the usefulness of these media for the isolation of these strains. Attempts have, therefore, been made to utilize other phenotypic characteristics for the differentiation of non-O157 on selective media (Gouali et al. 2013).

Posse et al. (2008) developed a set of novel differential media for the isolation and confirmation of *E. coli* O157:H7 and non-O157 strains (O26, O103, O111, and O145) from food and feces. The first differential medium for non-O157 strains is based on a mixture of carbohydrate sources, β -D-galactosidase activity, and selective reagents that result in a color-based differentiation of the four specified non-O157 STEC strains. The growth of the four different non-O157 STEC serotypes on this medium produces different colored colonies. *E. coli* O26 colonies appear as bright red to dark purple, O103 and O111 colonies are blue-purple, and O145 colonies are green. Suspect colonies are subsequently picked from the differential medium and streaked onto one of the more specific confirmation media. These agars

contain phenol red broth base supplemented with dulcitol, L-rhamnose, D-raffinose or D-arabinose (Mathusa et al. 2010). These agars have been reported to result in changes to the original stated colors according to incubation time, how crowded or isolated the colonies on the agars or the medium or food matrix which they are isolated from.

Rainbow® O157 agar has been evaluated as a selective media for the detection of non-O157 serotypes and produce different color reactions for different serotypes present (Mathusa et al. 2010). However, some strains of non-O157 serogroups, mainly from serogroup O103, is inhibited by the concentration of potassium tellurite used in Rainbow® O157 agar (0.8 mg/L) as well as CT-SMAC (2.5 mg/L) (Fukushima et al. 2000; Tillman et al. 2012). A modified version of Rainbow® agar (mRBA) containing 0.05 mg/L cefixime, 0.15 mg/L potassium tellurite, and 5 mg/L novobiocin that supports the growth of various STEC strains is described for the isolation of the *E. coli* and the “top six” non-O157 and recommended in the USDA FSIS Microbiology Laboratory Guidebook (MLG 5B.04) (USDA-FSIS 2013). The procedure also involves an IMS step and a subsequent acid treatment procedure which can reduce the growth of natural microflora while allowing acid-tolerant STEC to grow (Tillman et al. 2012).

CHROMagar™ was developed, and has been found, to allow the growth and presumptive identification (mauve colonies) of approximately 75% of STEC isolates in a vast collection of isolates comprising of 20–40 different serotypes, including the common serotypes of Enterohemorrhagic *E. coli* (EHEC) (Hirvonen et al. 2012; Tzschoppe et al. 2012). This media failed to detect only 5/249 from three published studies (Hirvonen et al. 2012; Tzschoppe et al. 2012; Gouali et al. 2013; Wylie et al. 2013). CHROMagar™ was found to have good performance in a clinical trial of stool specimens and was found to recover different STEC serotypes including non-sorbitol fermenting *E. coli* O157:H7 and O104:H4. The use of this media is also rapid, whereby putative STEC colonies can be clearly visualized the day after sample receipt, and performed well against the “gold standard” method of the laboratory (Gouali et al. 2013). A second medium (CHROMagar™ STEC), derived from the original medium, was developed to characterize *E. coli* O104:H4 following the European outbreak (Inset 1.2), and reported to identify the outbreak strain, which produced an extended-spectrum lactamase (ESBL), but not other sporadic *E. coli* O104:H4 isolates that did not produce ESBL (Gouali et al. 2013).

Enterohemolysin agar was developed based on the observation that many non-O157 and O157 strains produce a narrow zone of hemolysis on blood agar supplemented with the red blood cells of sheep and calcium ions after 18–24 h incubation (Beutin et al. 1989). However, a disadvantage of the media is that not all STEC show hemolysis on this agar and the non-selective properties of the agar allows for the growth of background flora present in the sample. Also enterohemolysin positive colonies must be screened for Stx production as some non-STECS that are alpha-hemolytic can interfere with interpretation of colonies (Grant et al. 2011). Supplementation with vancomycin (30 mg/L), cefixime (20 ug/L), and cefsulodin (3 mg/L) was found to be superior to enterohemolysin agar for the detection of hemolysis by STEC (Lehmacher et al. 1998).

2.3.2 Molecular Detection of Shiga Toxin-Producing *E. coli*

The World Health Organization (WHO) has called the rapid identification of virulent non-O157 STEC a public health priority (WHO 1998). Recent recommendations from the Centers for Disease Control and Prevention (CDC) suggest that future STEC methods should include an assessment of the potential of the organisms to cause severe disease, possibly by detecting virulence factor genes (Mingle et al. 2012). These recommendations also proposed that comparative genomic studies performed on existing and newly sequenced STEC strains may identify gene targets that will likely aid in the identification or predications of pathogenicity in the future (Gould et al. 2009; Mingle et al. 2012). A range of molecular techniques such as conventional polymerase chain reaction (PCR) (Paddock et al. 2012), RT-PCR (Fratamico et al. 2011), PCR coupled to mass spectrometry (Shen et al. 2013), and isothermal nucleic acid amplification (Wang et al. 2012) have been employed in the detection of gene targets associated with STEC strains.

The FDA-BAM method (Feng et al. 2011a) recommends a molecular screening step of food enrichments for *E. coli* O157:H7 and other STEC using an RT-PCR assay. Implementation of an initial screen test can offer time and cost benefits by reducing the number of samples requiring confirmation and the time that it takes to test. The assay detects the *stx*₁ and *stx*₂ genes and the +93 single nucleotide polymorphism (SNP) in the *uidA* gene that encodes for the β -D-glucuronidase enzyme (Feng and Lampel 1994; Jinneman et al. 2003). The SNP is highly conserved in *E. coli* O157:H7 strains that produce Stx and is an accurate marker for these pathogens (Feng 1993). This assay also detects other STEC strains where a +93 *uidA* negative but *stx*₁ and/or *stx*₂ positive result indicates the sample may contain an STEC, but not necessarily a pathogenic STEC (Feng et al. 2011a). It is therefore essential to isolate and confirm the pathogenic STEC for additional testing.

The USDA-FSIS method (MLG 5B.05) also involves performing a molecular screen of samples targeting specific virulence genes, followed by the isolation and confirmation of STEC from screen test positive broths. The targets utilized in MLG 5B.05 are also extensively used in research and commercial tests and include Shiga toxin encoding genes (*stx*), an attaching and effacing gene (*eae*), and genes specific for each of the Big 6 *E. coli* O serogroups. An RT-PCR test (BAX® System RT-PCR Assay—STEC Suite, Dupont) performed on a commercially available platform (BAX® RT-PCR system) is used to detect each gene and provide the user with an automated interpretation of the result. Any samples that test positive for all three gene targets; *stx*, *eae*, and an O antigen are considered potentially positive for a Big 6 STEC and sent for confirmation.

Numerous RT-PCR methods that use similar approaches to the FSIS protocol have been used for the sensitive detection of STEC in enrichments of ground beef, beef carcass swabs, beef trim, and a range of other food matrices such as apple juice and raw-milk cheeses (Wang et al. 2013). Clinical laboratories have also imple-

mented similar RT-PCR methods to detect the Big 6 STEC serogroups in samples, which has improved timeliness of identification and outbreak investigations (Mingle et al. 2012). In addition, a range of commercially available systems have been developed that utilize a host of different technologies and targets to detect the Big 6 or Top 7 STEC serogroups (Table 2.1).

While most commercial test systems rely on the detection of *stx*, *eae*, and O antigen targets, some utilize additional or alternative targets which may provide a better indication of the presence of a Big 6/Top 7 STEC. For example, the Atlas STEC EG2 Combo Detection Assay designed by Roka Biosciences (San Diego, CA, USA) uses an alternative target that is believed to eliminate false-positive results that may arise from co-contamination of samples with non-STEC organisms that possess *stx* and *eae*. Alternatively, the NeoSEEK™ (Neogen, Lansing, MI, USA) approach to STEC detection and identification employs a high throughput SNP genotyping platform to detect 70 independent targets. The number and range of targets employed is believed to provide sufficient evidence for the non-culture based confirmation of Big 6 STEC. In a modification to the FSIS method, the Assurance GDS system employs an IMS step to separate target O serogroups from enrichment cultures prior to cell lysis and PCR detection of STEC virulence genes by RT-PCR. Many of these commercial screening systems have been issued with a letter of no objection for testing beef products (USDA-FSIS 2014b), and/or have been validated by recognized independent bodies for the detection of STEC in other food types such as poultry, spinach, leafy green vegetables, and sprouts.

Despite their widespread popularity, detection systems that rely solely on *stx*, *eae*, and O antigen markers to detect STEC in complex matrices have a number of limitations associated with these gene targets. Beef cattle feces and beef trim enrichments represent two examples of complex matrices that can contain multiple isolates of *E. coli* harboring different combinations of genetic markers (*stx*, *eae*, and O antigen genes) that are not all associated with a single isolate. Isolates meeting this description, while not considered a Big 6 STEC, are likely to contribute to the potential positive status of a sample. To further confound screening protocols, *stx* and *eae* gene targets may also be harbored by non-Top 7 STEC and bacterial species other than *E. coli* (Schmidt et al. 1993; Paton and Paton 1996; Karch et al. 1999; Gyles 2007; Chandry et al. 2012), further contributing to the potential positive status of a sample. Samples may also contain free *stx*-phages that interfere with the detection of STEC (Martinez-Castillo and Muniesa 2014), and *stx* specific primers may not detect all *stx* variants (Feng et al. 2011b). Therefore, any method that solely rely on *stx*, *eae*, and O antigen targets to screen for Top 7 STEC in complex matrices should confirm the presence of pathogens in screen test positive samples. If no attempt is made to confirm screen positive samples, then those samples are considered positive for a Top 7 STEC serogroup by the FSIS (USDA-FSIS 2014c). Since studies have reported a large number of potential positive samples that cannot be culturally confirmed (Bosilevac and Koohmaraie 2012), failure to confirm samples could result in substantial cost to industry.

Table 2.1 Selected commercially available kits for the detection of Shiga toxinogenic *Escherichia coli*. (Adapted from Wang et al. 2013; USDA-FSIS 2014b)

Name of test	Supplier	Target STEC serogroups	Test type/technique	Recommended/validated samples types ^a
ACTERO <i>Salmonella</i> STEC Enrichment Media	FoodChek System Inc.	Top 7 STEC	Magnetic immunochromatographic technology (lateral flow)	Raw ground beef, liquid whole egg, raw ground chicken, raw frozen scallops, sprouts, environmental surfaces (stainless steel, plastic, rubber, ceramic tiles, sealed concrete)
Atlas STEC EG2 Combo Detection Assay	Roka Bioscience, Inc	Top 7 STEC	Target capture of nucleic acid, Transcription-Mediated Amplification and Hybridization Protection Assay	Fresh raw ground beef (73 % lean), fresh raw beef trim, romaine lettuce
Assurance GDS MPX Top 7 STEC	BioControl Systems	Top 7 STEC	Immunomagnetic capture and RT PCR	Raw beef trim, raw ground beef, raw spinach, and raw mixed greens (baby lettuces and other leafy green vegetables)
BAX® System real-time PCR STEC suite	DuPont Qualicon	Big 6 STEC—O26, O45, O103, O111, O121, O145	Real-time polymerase chain reaction (RT PCR)	Raw beef trim, raw ground beef, raw ground beef plus soy
GeneDisc Plate STEC Top 7	Pall GeneDisc Technologies	Top 7 STEC—O26, O45, O103, O111, O121, O145, O157	RT PCR	Fresh raw ground beef (20% fat), fresh raw beef trim (20% fat)
IEH <i>E. coli</i> O157 (Stx-producing <i>E. coli</i> (STEC) with Intimin and <i>Salmonella</i> Test System	IEH Laboratories & Consulting Group	Top 7 STEC	RT PCR	Raw ground beef, raw beef trim, raw poultry, RTE turkey (omitted for STEC) and mixed leafy greens
iQ-Chek™ STEC VirX and iQ-Chek™ STEC SerO	Bio-Rad Laboratories	Top 7 STEC	RT PCR	Raw beef trim
NeoSeek STEC confirmation	Neogen Corp.	Big 6 STEC	PCR mass spectrometry	Ground beef trim

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