Policy on the Control of *E. coli* O157:H7 Contamination in Raw Beef Products

Effective date:

This policy is effective on the date of publication and all operators of registered establishments handling raw beef are required to reassess their HACCP system or their process controls (See point 1), implement pathogen reduction step(s) if not already in place, validate their HACCP system and implement a verification procedure.

Policy:

In light of available information on the prevalence of *E. coli* O157:H7 in bovine animals showing levels higher than initially thought, establishments producing or receiving raw beef are required to reassess their HACCP system to take into consideration the hazard associated with *E. coli* O157:H7 in raw beef. For the purpose of this policy, the term raw beef includes veal, hearts, head meat, cheek meat, oesophagus, etc. (i.e., striated muscle) but excludes beef tails and tongues since these are customarily fully cooked and have not been linked to human illnesses. Raw beef includes intact and non-intact raw beef. An **INTACT** raw beef cut is a piece of meat for which the integrity has not been modified i.e., bacteria are on the surface and interior is likely sterile. **NON-INTACT** raw beef is beef that has been either needle-tenderized, injected, diced or ground.

1. Operators of all establishments that produce or receive raw beef products for processing are required to **reassess their HACCP system** to determine whether *E. coli* O157:H7 contamination is a hazard likely to occur in the production of these products, and, if so, whether their HACCP system appropriately addresses this hazard. In the absence of a HACCP system at the establishment, the Operator is required to develop and implement a documented, validated and auditable HACCP-based process controls to address this specific hazard. For the purpose of this section, the term HACCP system encompasses all process controls that need to be used by the operator to control the *E. coli* O157:H7 even if not yet FSEP recognized.

2. It is clear, based on available information, that *E. coli* **O157:H7 contamination of raw beef is a health hazard likely to occur** and that control measures need to be implemented. However, if an operator has concluded, as a result of the reassessment, that the *E. coli* O157:H7 hazard associated with raw beef products produced at the establishment is unlikely to occur, the determination made will have to be supported by scientific evidence (e.g., scientific literature, data, etc.). The information will be reviewed by the CFIA in light of all scientific information available. It should be kept in mind that *E. coli* O157:H7 is considered to be an adulterant in ground beef in the USA and subject to recall when positive results are found in Canada. Further, intact or non-intact raw beef products may be used to produce raw ground beef (See introduction for the definition and paragraph 11 for more information on intact beef products). The objective of these requirements is to ensure that the appropriate control measures are taken through a thorough reassessment of the establishment's HACCP system.

3. When the operator has concluded, as a result of the reassessment, that the *E. coli* O157:H7 hazard associated with raw beef products produced at the establishment is likely to occur, **FSEP forms (or equivalent)** on product identification and intended use are to be reviewed for completeness and accuracy. The *E. coli* O157:H7 hazard shall be clearly identified on the FSEP form # 5. Then, this hazard needs to be passed through the decision tree (FSEP form # 8). The operator has to decide how the hazard will be addressed either through CCP(s) at the establishment during production, when receiving raw beef products (letter of guarantee from the suppliers) or through other measures that will prevent distribution of potentially contaminated products. The operator must ensure that all aspects of the establishment's HACCP system have been updated as required.

4. Operators shall ensure that the level of *E. coli* O157:H7 in beef products distributed outside of federally registered establishments is **below detectable level** (i.e., no *E. coli* O157:H7 detected in a sample when tested with one of the Health Canada official screening methods (e.g. MFLP-81 (BioControl Assurance), MFLP-87 (BioControl VIP), MFLP -94 & 95 (Reveal 8 hours and 20 hours) and MFLP-30 (Dupont BAX). Confirmation tests, for the samples found positive (presumptive) with the screening test, will be done using the attached Immunomagnetic Separation (IMS) method). In all cases, control measures must be in place at the establishment to prevent growth of or contamination with *E. coli* O157:H7.

   The following control measures are to be implemented:

   a) In the case of a **slaughter establishment**, as a result of the reassessment of the establishment HACCP system, the operator shall use one or more validated intervention(s) (such as steam pasteuriser, organic acid sprays, etc. validated according to this policy) at the time of slaughter to reduce the contamination with *E. coli* O157:H7 to below detectable level.

   b) In the case of an **establishment receiving raw beef**, as a result of the reassessment of the establishment's HACCP system, the operator may:

      1. implement purchasing specifications and determine that a CCP is required at the receiving step to ensure that these purchase specifications are met. The specifications must require that all suppliers have one or more validated CCPs in their production of raw beef shipped to their establishment and that their controls are effective and ensure that *E. coli* O157:H7 is below detectable level.

      2. determine that validated CCP(s) is(are) already in place at the establishment or will be added to control the hazard associated with *E. coli* O157:H7 (e.g., all raw beef received is used in the production of fully cooked product).

   c) In the case of an establishment that produces raw beef that will be used **exclusively** in the manufacturing of fully cooked products in other federally registered establishments, the operator may determine, as a result of the reassessment of the establishment's HACCP system, that the hazard related to *E. coli* O157:H7 is likely to occur, but that no new CCP(s) is/are required in the establishment because all products are shipped to another federally registered establishment where steps are taken to control the hazard. The operator must then provide details on the control measures that will be implemented e.g., the product is sent under company seal to designated federally registered establishment and processed as prescribed (the operator of the receiving establishment provides a letter of guarantee to the effect that the cooking process will control the hazard), the product is clearly labelled e.g., "*Product destined for further processing in a fully cooked product*". Procedures must include monitoring, verification and deviation procedures, as well as record keeping, and be auditable and effective.

5. When the operator decides that one of the control measures to be taken at the establishment involves **purchase specifications**, the following points must be considered and filed accordingly:

   a) **A letter of guarantee** from supplying companies must be on file identifying interventions or other measures used to reduce, prevent or eliminate the hazard associated with *E. coli* 0157:H7. The letter must be dated and signed by the operator, or a designated person, of the supplying establishment. The letter must include a statement to the effect that when the supplier interventions have been found to be ineffective and that a positive result is obtained, the CFIA inspector of the receiving establishment and all establishments that received implicated product will be notified. Note: When implicated product has been distributed, the Office of Food Safety and Recalls (OFSR) must be notified (see also paragraph 12).

b) As a **verification step for the receiving CCP**, the receiving establishment must verify that the supplier interventions are effective through random testing incoming product, at a frequency determined by the Operator. That frequency should be based on the establishment knowledge of their suppliers' ability to meet their purchasing requirements. An acceptable alternative to verification testing at receiving could be to require certificates of analysis from suppliers that demonstrates that the product is below acceptable level. These analyses should be done in an independent laboratory using official methods.

Note: A certificate of analysis should not be misconstrued with a letter of guarantee. A letter of guarantee provides confirmation that the process under which the product was manufactured is under control and that the purchase specifications are met. A certificate of analysis provides additional information about testing results for a specific lot. A certificate of analysis, although providing an increased level of confidence, cannot be used in lieu of a letter of guarantee.

6. In the case of establishments handling both raw beef products considered to be below detectable level for *E. coli* O157:H7 and beef products potentially contaminated with *E. coli* O157:H7, the operator must develop and implement a written segregation protocol considering the status of the different products. The appropriate information must be reflected on FSEP forms (Potential cross-contamination). The segregation procedures must include monitoring, verification and deviation procedures as well as record keeping, and be auditable and effective. For example, a letter of guarantee regarding *E. coli* O157:H7 is required only for products received for raw beef product manufacturing and not for those received for further cooking. Segregation procedures must be implemented to ensure that raw beef products received for cooking are not used in the production of finished raw beef products and that cross-contamination is prevented.

7. The measures taken under HACCP systems should be elaborated under established FSEP guidelines. When *E. coli* O157:H7 is detected, the operator must reassess the establishment HACCP system, take corrective measures and implement preventative measures (See paragraph 13).

When an establishment has determined through the reassessment of its HACCP system that control measures other than CCPs are required to control the *E. coli* O157:H7 hazard (see paragraph 4c), those other measures must include monitoring, verification and deviation procedures as well as record keeping, and be auditable and effective.

8. Validation of in-plant pathogen reduction steps (CCPs) must be performed as per the FSEP approach. FSEP defines validation as: obtaining confirmation that the elements of the HACCP system are complete and effective in controlling biological, chemical and physical hazards. This may include ingredient sampling, end product sampling, etc. In this case, this means more specifically doing all of these 3 steps:

Step 1 gather published scientific information on the experimental reduction effect of the chosen pathogen reduction intervention and all relevant critical factors (for example, intervention "Y" should result in a 2.0 log reduction considering parameters defined under experimental conditions such as pressure, temperature, time, chemical concentration, etc...);

Step 2 demonstrate the effectiveness in reducing a suitable surrogate level by "X" logs under in-plant operational conditions (e.g., generic *E. coli* or *Enterobacteriaceae* as indicators are reduced by "X" logs by intervention "Y") for each of the interventions that the establishment has chosen to implement. An appropriate surrogate is an organism that has a heat resistance, growth range, pH range, ability to grow on selective media, etc. that is similar to *E. coli* O157:H7. This is normally done by comparing surrogate levels in a sample taken **before and after** the intervention. The operator shall choose a statistically significant sample size over a period of **4 months** to demonstrate that on-site intervention is achieving the log reduction targeted. ***E. coli* O157:H7 must not be introduced for experimental purpose in registered establishments** (See Appendix 4 for guidance on sample size and statistical analysis). Sampling of carcasses should be conducted in accordance with Annex T, USA section, Chapter 11 of the Meat Hygiene Manual of Procedures.

Step 3 the sum of the log reductions from all interventions (CCPs) must result in a final product that is below detectable level for *E. coli* O157:H7. The demonstration should be based on a statistically significant number of finished product samples i.e., provide a 95% level of confidence that *E. coli* O157:H7 is below detectable level. Because of the expected variability of *E. coli* O157:H7, this validation sampling should be done by randomly taking the required number of samples **over one month** of production as reflected in Table 1. The minimum acceptable level of sampling required by the CFIA will be the 1% threshold column. Sampling of carcasses should be conducted in accordance with Annex T, USA section, Chapter 11 of the Meat Hygiene Manual of Procedures.

Note 1: If the lot size (i.e., number of animals slaughtered per month) falls between two values appearing in column 1 of Table 1, the highest number should be selected in order to determine the sample size.

Note 2: Exceptionally, when an approved processing method, such as fermentation, cooking and canning, is already recognized for its lethality and is implemented as per regulatory requirements, additional validation activities at the establishment level, as described above, are not necessary. Other processes may be exempted subject to prior evaluation by Headquarters.

Note 3: It is up to each individual company to decide if they want to use an accredited laboratory or not when testing for validation or verification purposes. In all cases, laboratories MUST use a Health Canada official method (See point 4 of this section). Laboratory results (laboratory tests certificates) shall indicate which method has been used to analyse the samples.

Validation is to be completed initially to demonstrate that the interventions put in place by the operator are effective in producing meat products that are below detection level for *E. coli* O157:H7. Validation has to be conducted again when the operations have changed and/or pathogen interventions have been modified/added. When a sample of product has been found positive to *E. coli* O157:H7, the whole HACCP system must be reassessed, and once the necessary changes have been implemented, at least the 3rd step of the validation must be conducted to demonstrate that the establishment is in control.

9. Verification must be performed as per the FSEP Program. In this case, this means more specifically that :

   a. records are reviewed;
   b. on-site reviews are conducted to ensure that the written program is implemented as planned;
   c. appropriate level of random product testing is done on a routine basis for *E. coli* O157:H7, or certificate of analysis received;
   d. audit of suppliers (Optional) may also be performed as a verification activity.

   No set frequencies are prescribed for these activities. It is the operator's responsibility to implement these activities at a frequency which will ensure that finished product will meet applicable requirements.

   Note: Certificates of analysis provided by suppliers (performed in a 3rd party independent laboratory) can be used in lieu of receiving establishment testing verification activities.

10. The following guidelines should be used to define a lot when a carcass, trimmings, manufacturing beef or ground beef are found positive to *E. coli* O157:H7 as a result of testing activities:

    a) For ground beef, refer to Health Canada guideline #10 (http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/rfao-aoca/e_guidelines_for_raw_ground_beef.html).

b) For carcasses, trimmings, or manufacturing beef, CFIA will refer to Health Canada guideline #10. CFIA would recognize the establishment's definition of the sampled lot, provided the establishment has a sampling plan and scientific basis supporting the definition of the sampled lot. However, CFIA cautions that the defined lot size does not exempt an establishment from its responsibility to consider whether there are connections between lots. For example, if multiple lots of beef trim were produced from source materials from the same production lot of a single supplier, and some of this product was found positive for *E. coli* O157:H7, CFIA would expect the establishment to have a scientific basis that justifies why any trim produced from those source materials should not be considered to be contaminated. If no satisfactory scientific basis is provided by the operator, the default lot consider by CFIA will be from clean-up and sanitation to the next clean-up and sanitation.

c) Similarly, with regard to carcasses, if one carcass was found positive for *E. coli* O157:H7, CFIA would expect an establishment to have a rationale explaining why other carcasses being produced on the same day, on the same line, are not contaminated with *E. coli* O157:H7 as well.

It should be noted that if an operator has a validated HACCP system and regularly tests specific lots of product and are found negative for *E. coli* O157:H7, this information could possibly be a basis for determining whether one *E. coli* O157:H7-positive lot will implicate other lots produced on the same day.

11. It should be noted that raw intact beef product used as such by the consumers does not pose the same level of risk as non intact product. In contrast to non-intact raw beef, the interior of intact raw beef products is considered free of pathogens. Consequently, customary cooking of these products will destroy any *E. coli* O157:H7 that might be present on surfaces. Operators will have to determine if all intact raw beef produced at the establishment will remain intact until consumed (e.g., pre-packaged steaks), or if it is possible that the product can be used in the production of non-intact raw beef products (e.g., primary cuts from which trimmings can be used to produce ground beef at retail). All non-intact raw beef must be either produced under controls to ensure that the products do not contain detectable levels of *E. coli* O157:H7, or to be used in the production of fully cooked ready to eat products under a HACCP plan that addresses the *E. coli* O157:H7 contamination hazard in a federally registered establishment. Like all establishments producing raw beef products, an establishment producing and distributing intact raw beef, e.g., steak, is required to reassess its HACCP system for this product in light of relevant data on *E. coli* O157:H7 to determine whether its HACCP system for this product appropriately addresses this hazard. An establishment producing and distributing pre-packaged intact steaks may conclude it does not need to change its HACCP system for these products.

12. With the exception of cases covered in point 4c), when an establishment receiving raw beef is in turn supplying raw beef products to another establishment, the following guidelines apply:

a) both establishments must have purchase specifications to prevent *E. coli* O157:H7 from entering their facility, including a sampling protocol for *E. coli* O157:H7 testing, as part of their verification activities. In this case, the letter of guarantee provided by the establishment that receives and ship raw beef should state that:

- the establishment has a CCP to control the hazard associated with *E. coli* O157:H7 at receiving and has letters of guarantee from all of their own suppliers on file;

- control measures are in place to prevent the growth or contamination by *E. coli* O157:H7 at the establishment once the product is received.

b) in addition to purchase specifications addressing *E. coli* O157:H7, receiving establishments must ensure that measures are in place to prevent *E. coli* O157:H7 growth or contamination after product receipt through their prerequisite programs. In this case, no additional finish product testing is necessary for verification purpose.

For other information on letter of guarantee, please refer to paragraph 5.

13. Positive results for *E. coli* O157:H7 as a result of validation or verification activities:

Before taking a sample for *E. coli* O157:H7 testing, the operator must isolate and clearly identify the lot to the satisfaction of the CFIA inspector in order to ensure that the product is not incorporated into a finished raw beef product. It is recommended that the lot be detained pending until laboratory results are received. The operator must further identify the supplying establishment number (if product received from another establishment), the production date, production lot number and any other relevant data available about the lot.

If positive or presumptive positive results are received, the operator must immediately hold the lot and prevent its use. If confirmed, the operator must also immediately notify the supplier of that lot and the CFIA responsible inspector. The product will have to be disposed of either by fully cooking the product or by condemning the product. When the product is found positive to *E. coli* O157:H7, the responsible inspector will follow-up on the necessary reassessment activities and will notify the Chief Program Network, (Food of Animal Origin) which will notify the responsible inspector of the supplying establishment. If the product was imported, the Area Office will immediately notify in writing the Chief, Import Programs, Food of Animal Origin Division, Ottawa. The Chief Import Programs will notify the authorities of the exporting country for a follow-up investigation. In summary there are three possibilities :

a) **In the case where a presumptive or a positive result is obtained as a result of validation or verification testing on products from the establishment**, the operator must take those results as evidence that their HACCP system has been ineffective in producing a product that is below detectable levels. Consequently, the operator must immediately notify the responsible inspector and take immediate corrective actions, including continue to hold the product to prevent its use, to dispose of the product appropriately (fully cooked or condemned and denatured), to reassess their HACCP system to determine the reason for the deviation, and to determine what step(s) is/are necessary to prevent a recurrence of the deviation. Serious consideration should be given to increase the lethality of the pathogen reduction steps used in the HACCP system. After the changes have been implemented, the system must be re-validated by taking the required number of samples over a complete month of production (i.e., Step 3 of the validation, see paragraph 8). The adequacy of the operator's corrective and preventive actions will be verified by the CFIA as soon as possible through a system audit or other inspection activities (when not FSEP recognized) and a review of the validation data. The applicable elements of the HACCP system will be targeted in the audit scope.

b) **In the case where presumptive or positive result is obtained as a result of verification testing done on products received by a specific establishment**, the operator must immediately notify the responsible inspector and take immediate corrective actions, including continue to hold the product to prevent its use and to dispose of the product appropriately (fully cooked or condemned and denatured or return to supplier under government seal). In addition, the operator must contact the supplying establishment to inform them of the test results. The supplying establishment must take this notification as evidence that their HACCP system has been ineffective in producing a product that is below detectable level (see above paragraph for actions to be taken by the operator of the supplying establishment and by the CFIA).

c) **In the case where the product found positive (or presumptive positive) to *E. coli* O157:H7 contains materials from various establishments and that the exact source of contamination cannot be identified (e.g., finished product testing)**, the operator must immediately notify the responsible inspector and take immediate corrective actions including, continue to hold the product to prevent its use and to dispose of the product appropriately (fully cooked or condemned). In addition, the operator must notify all the suppliers that their product was potentially contaminated. The purpose of the notification is to ensure the supplying establishments know that they could be the source of *E. coli* O157:H7. Each of the suppliers must review their HACCP system for the implicated lots to determine whether any evidence exist that the HACCP system was not implemented properly. HACCP system reassessment with appropriate corrective and preventive measures may or may not be warranted depending on the findings. The decisions of the establishment in this regard should be evaluated by the CFIA.

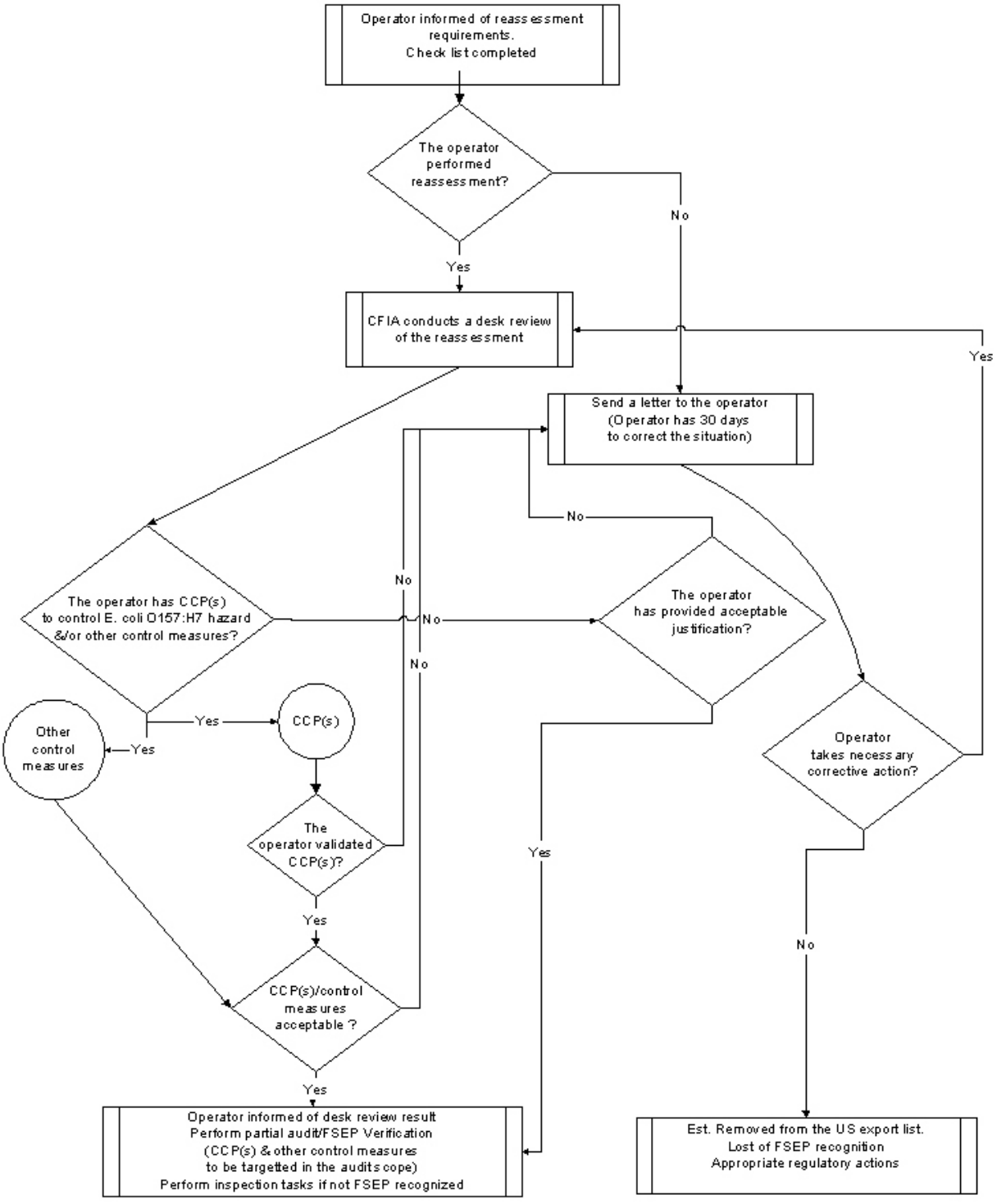
Depending on the circumstances, the options for product disposition, which must be conducted under CFIA's authorization and supervision, are as follows:

- process the product into a fully cooked finished product (if done in a different establishment, must be transferred under official government seal and Form # CFIA/ACIA 3256);
- condemn and denature the product under CFIA's supervision;
- in the case of a product received from another registered establishment, reject and return the product to the supplier under official government seal and CFIA/ACIA 3256 for appropriate disposition.

14. CFIA will conduct a systematic review (see Appendix 1 - *E. coli* O157:H7 reassessment review flowchart) of operator's reassessment to evaluate the acceptability and effectiveness of the measures taken to comply with this policy. As part of the process, the CFIA responsible inspector will complete the check list (see Appendix 2 - Check list for the review of establishment *E. coli* O157:H7 reassessment) and confirm that the information supplied by the Operator (e.g., HACCP Coordinator) is complete and reflects in-plant conditions.

    In cases where deficiencies are found, the operator will be informed in writing by the FSEP Coordinator of the deficiencies identified and will be required to identify and implement appropriate corrective measures and resubmit a revised reassessment package within 30 calendar days of the issuance of the letter. Since validation data will need to be collected over a period exceeding the 30 day period as part of the new reassessment, an additional delay will be granted by the Program Network Chief, Food of Animal Origin after assessment of the establishment's written validation protocol. A tracking report is to be submitted monthly to the National Manager Meat Programs by the Program Network Chief, Food of Animal Origin. This report should include all Area establishments handling raw beef, along with their reassessment status (See Appendix 3). Operators failing to comply with these requirements will lose their privilege to export to the USA and, if FSEP recognized, steps will be taken to withdraw their FSEP recognition as per established FSEP procedures. As part of a risk-based approach and to mitigate the risk to consumers, the CFIA headquarters may also increase their product sampling and testing for *E. coli* O157:H7 in those establishments that have failed to comply with these requirements. Plant rating may also be lowered accordingly.

Appendix 1: E. *coli* 0157:H7 Reassessment Review Flowchart



Appendix 2: Checklist for the Review of Establishments *E. coli* O157:H7 Reassessment

Establishment Number: . . . . . **Name:** . . . . . .

Inspector/Veterinarian-In-Charge:

. . . . . .

Part A:

		Yes	NO
Q1	Does the establishment receive or produce raw beef products, including intact beef products (e.g. carcasses, quarters, cuts, ...) or non-intact beef products (e.g.:ground beef, MSM, FTM, tenderized beef, etc.)? If NO stop here, if yes Continue to Q2..	❑	❑
Q2	Is the establishment eligible to export to the United States?	❑	❑
Q3	Has the size of the establishment been confirmed? If so, is it **Large, Medium, Small. (Please circle the size that applies.)**	❑	❑
Q4	Has the establishment completed the reassessment of its HACCP system in view of the new data on *E. coli* O157:H7? **Date:** . . . . . Identify HACCP plan(s) concerning raw beef products received and/or produced. 1. . . . . . 2. . . . . . 3. . . . . . 4. . . . . . 5.	❑	❑
Q5	How frequently does the establishment review and update its HACCP system? -annually (minimum acceptable) -when changes are made in the establishment that necessitate hazard re-analysis?	 ❑ ❑	 ❑ ❑

Date reviewed by the Inspector/ Veterinarian-In-Charge: . . . . . **Date sent to Area Office:** . . . . . .

Signature of Inspector/Veterinarian-In-Charge:

. . . . . .

Comments:

. . . . . .
. . . . . .
. . . . . .
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❑ Acceptable to Area Office

Date:

. . . . . .

Signatures :

Program Specialist:

. . . . . .

FSEP Coordinator:

. . . . . .

Establishment Number: . . . . .

Part B: Complete for <u>each</u> HACCP plan identified in Part A- Q4.

HACCP Plans: . . . . . .

		Yes	NO
Q1	Has the establishment specifically identified *E. coli* O157:H7 as a hazard on Form #5? e.g.: Raw beef products: Presence of *E. coli* O157:H7 in raw product. Screening of suspect animals: Contamination by *E. coli* (including *E. coli* O157:H7) or other pathogen(s) through contact with soiled animals during evisceration. Skinning: Cross-contamination of carcass by *E. coli* (including *E. coli* O157:H7) or other non-sporulating pathogen(s) during skinning (due to fecal contamination and/or to contact of skinned portion with hide). Tying of esophagus or bung tying: Contamination by *E. coli* (including *E. coli* O157:H7) or other pathogen(s) due to poor working techniques. Evisceration: Contamination by *E. coli* (including *E. coli* O157:H7) or other pathogen(s) due to poor working techniques. Trimming: Presence of pathogen(s) (including *E. coli* O157:H7) due to inadequate trimming of contaminated area and/or inadequate trimming techniques. Final shower: Failure to remove bacteria weakly adhering to external and internal surfaces. Washing and application of anti-microbial agents (organic acid sprays, chlorine, chlorine dioxide and acidified chlorine solutions): Survival of pathogen(s) (including *E. coli* O157:H7) due to inadequate disinfection. Carcass pasteurizing: Survival of pathogen(s) (including *E. coli* O157:H7) due to inadequate procedures. Grinding/MSM/FTM/Tenderizing: Introduction of pathogen(s) (including *E. coli* O157:H7) in intact product during processing to non-intact product. Cooking: Survival of pathogen(s) (including *E. coli* O157:H7) due to inadequate procedures (time/temperature). Process Steps: Re-contamination by pathogen(s) (including *E. coli* O157:H7) or surface growth due to inadequate cooling.	❑	❑
Q2	Have the hazards identified on Form #5 been carried over to Form #8 and has the hazard determination been performed by using the decision tree? NB: Prerequisite Programs do not reduce hazards associated with *E. coli* O157:H7 to an acceptable level.	❑	❑
Q3	When answering Q2 on Form #8, with regard to *E. coli* O157:H7 is it thought "likely that contamination with the identified hazard could occur in excess of the acceptable level or could increase to an unacceptable level?" **If E. coli O157:H7 has been identified as a hazard on Form #5 this answer should normally be YES.**	❑	❑
Q4	Has the establishment implemented a CCP using the decision tree?	❑	❑
Q5	Has the establishment previously implemented any actions to address *E. coli* O157:H7?	❑	❑
Q6	Has the establishment implemented additional actions to address *E. coli* O157:H7?	❑	❑

Establishment Number: . . . . . .

Q7	If no further actions were taken, have you received a written rationale from the establishment outlining why no actions were taken? -Stop here and issue a 30 day letter if the establishment has implemented no actions to address *E. coli* O157:H7 and has provided no rationale. -Forward rationale and all documentation to the Director, Food of Animal origin Division at **H.Q.** Comments of Inspector/Veterinarian-In-Charge (attach Appendix if necessary):	❑	❑
Q8	A. Does the establishment have evidence that all its suppliers of raw beef have implemented one or more CCPs to bring the incoming product below detectable level for *E. coli* O157:H7, and is the establishment conducting microbiological verification testing of incoming raw beef (including imported product, if any) or receives certificate of analysis from suppliers (analysis done in an independent laboratory with official methods - see CFIA Policy on *E. coli* 0157:H7)? If not, proceed to B **and Q9 must be YES** (b) If a hazard of *E. coli* O157:H7 in incoming raw material is controlled at a process step other than Receiving, are all products subjected to this control at that same process step?	❑ ❑	❑ ❑
Q9	Has the establishment implemented one or more CCP(s) to address *E. coli* O157:H7? **If NO Q8-B must be YES** Specify: ❑ CCP - Receiving ❑ CCP - Screening ❑ CCP - Evisceration ❑ CCP - Trimming ❑ CCP - Washing ❑ CCP - Carcass pasteurizing ❑ CCP - Cooking ❑ CCP - Irradiation (when approved) ❑ CCP Fermentation Other CCPs: specify:	❑	❑
Q10	Have acceptable critical limits been defined for each CCP identified in Q9?	❑	❑
Q11	Have acceptable monitoring procedures been described and implemented for each CCP identified in Q9?	❑	❑
Q12	Have acceptable deviation procedures for non-complying or potentially non-complying product been described and implemented for each CCP identified in Q9?	❑	❑
Q13	Have acceptable deviation procedures aiming to eliminate the cause of the problem or its recurrence (preventive measures) been described and implemented for each CCP identified in Q9?	❑	❑
Q14	Have acceptable verification procedures been described and implemented?	❑	❑
Q15	Do verification procedures include microbiological verification testing to ensure the effectiveness of existing procedures (letters of guarantee from suppliers or in-plant CCPs)?	❑	❑
Q16	Has the establishment included an in-plant validation of their control measure or CCPs for the control of E. coli O157:H7?	❑	❑
Q17	Has the establishment provided test results of their validation (microbiological tests)?	❑	❑
Q18	Is the establishment using statistically significant (confidence level = 95%) sampling methods for **VALIDATION** of measures to address *E. coli* O157:H7?	❑	❑

Establishment Number: . . . . . .

The following documents must be sent to the Area Office FSEP Coordinator (the word “Confidential” must be marked on the envelope and on the documents):

- HACCP Plan(s) for beef products; (include the complete HACCP Plan(s) and indicate where the changes were made)

- **CHECKLIST FOR THE REVIEW OF ESTABLISHMENTS *E. COLI* O157:H7 REASSESSMENT**;

- Results (data) supporting in-plant validation of HACCP Plans (See Canadian Policy on the control of *E. coli* O157:H7 hazard in raw beef, section 8.)

- Written rationale provided by the establishment if no further actions were taken to address *E. coli* O157:H7.

Appendix 3 Monthly Status Report on E. coli O157:H7 Reassessment

Area: _____

Est. handling beef (Est. No)	Operators Reassessment Completed (date)	CFIA Desk Review Completed (date)	Validated CCP(s) or other measures In Place (Y/N)	30 Day Letter (date issued)	Comments	Additional delay granted. Due date (date)	Satisfactory On-Site Verification Audit Completed	Evaluation of Reassessment Completed (date)

Appendix 4 Number (n) of beef carcasses to select for the validation of microbiological reduction interventions based on *Enterobacteriaceae* (EB) counts

N	Group 1	Group 2	Group 3
	n for each Group		
100000	15	133	237
50000	15	133	236
25000	15	133	235
10000	15	132	231
5000	15	130	226
1000	15	118	192
500	15	105	161
100	13	57	71
50	12	37	41
25	10	21	23
10 or less	6 or less	9 or less	10 or less

Confidence interval +/- 0.5 log on the difference of EB log counts with 95% Confidence Level

n = Number of samples to be taken over a 4-month period according to the 4-month production volume (N)

N = Production volume over 4 months

Group 1 = Standardized intervention i.e. fully automated commercial equipment with monitoring devices e.g. steam pasteurizers

Group 2 = Intervention that is not fully automated i.e., equipment involving some manual intervention or not having monitoring devices for all parameters e.g., steam vacuum, organic acid sprays, etc...

Group 3 = New interventions and all other interventions not in group 1 or 2

Sampling Protocol

The sampling should encompass a 4-month period. It is recommended to sample during the seasons having the highest prevalence level.

Carcasses should be randomly selected over the validation period. Days, and hours within a day, should be determined in advance to create a sampling plan . At collection time, carcasses should be selected in a blind manner (e.g. 5th carcass following a carcass purposely selected). Side A (right or left) should be sampled for EB evaluation before the intervention of interest. Side B (left or right) of the same carcass should be sampled after the intervention. For the next carcass identified in the sampling plan, side B should be sampled first (before) and then side A (after). As a principle, the selection of side A or B should be alternated from one selected carcass in the sampling plan to the next one for the EB evaluation before the intervention of interest.

Statistical Evaluation

To assess the efficacy of the reduction intervention beyond chance, it is recommended to use a paired t-Test. This test is available in Excel (as an Add-Ins) under Tools / Data Analysis / t-Test: Paired Two Samples for Means. The Input Variable 1 Range should correspond to before values of EB counts. The Input Variable 2 Range should correspond to after EB counts. The Hypothesized Mean Difference should be set to 0, and Alpha to 0.05. Successful interventions should result in a P value inferior to 0.05.

Table 1: Sample size to obtain a 95% confidence level that the product will be below 1%, 0.5% or 0.1% of E. coli O157:H7 during a one month period

Lot size (No slaughtered per month)	Sample size (1.0% threshold)	Sample size (0.5% threshold)	Sample size (0.1% threshold)
10 - 69	All	All	All
70	69	All	All
80	78	All	All
90	87	All	All
100	95	All	All
110	103	All	All
120	110	119	All
130	117	129	All
140	123	138	All
150	129	147	All
160	135	156	All
170	141	165	All
180	146	174	All
190	150	182	All
200	155	190	All
300	189	259	All
400	210	310	All
500	224	349	499
600	235	378	596
700	243	402	690
800	249	421	781
900	254	437	868
1000	258	450	950
1100	261	461	1028
1200	264	471	1101
1300	266	479	1170
1400	268	486	1235
1500	270	493	1296
1600	272	499	1354
1700	273	504	1408

Lot size (No slaughtered per month)	Sample size (1.0% threshold)	Sample size (0.5% threshold)	Sample size (0.1% threshold)
1800	275	508	1459
1900	276	513	1507
2000	277	517	1552
2100	278	520	1595
2200	279	523	1636
2300	280	526	1674
2400	280	529	1711
2500	281	532	1745
2600	282	534	1778
2700	282	536	1809
2800	283	538	1839
2900	283	540	1867
3000	284	542	1894
3200	285	545	1945
3500	286	549	2012
3800	287	553	2072
4200	288	557	2141
4600	289	560	2201
5100	290	564	2265
5700	290	567	2329
5800	291	568	2339
6700	292	572	2415
7900	293	576	2492
9700	294	580	2576
12 400	295	583	2660
17 200	296	587	2748
28 200	297	591	2841
77 300	298	595	2937
100 000	300	596	2950
125 000	300	600	2959
Over 125 000	300	600	3000

Appendix 5

USDA
Microbiology Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG 5.03

Title: Detection, Isolation, and Identification of *Escherichia coli* O157:H7 and 0157:NM (Nonmotile) from Meat Products

Effective Date: 10/25/02

Description and purpose of change(s):

The Microbiology Laboratory Guidebook method chapters are currently under revision. The formatting is being changed to meet the requirements of the laboratory's document control system. Additional content is being added, i.e. Section 5.1.2. Limits of Detection, to meet the requirements of ISO 17025. Safety Precautions are also included in the revised chapters.

QD-F-Micro-0004.00 Approved: B. Cottingham, 4/18/02

Approved: Phyllis Sparling, 10/17/02

Procedure Outline

5.1 Introduction

5.1.1 General

The following method is used for the analysis of raw and ready-to-eat meat products for *Escherichia coli* 0157:H7 and 0157:NM (0 157:H7/NM). The method is based on enrichment in a selective broth medium, application of a rapid screening test, immunomagnetic separation (IMS) in paramagnetic columns, and plating on a highly selective medium.

The following definitions are used for reporting purposes. A potential positive sample causes a positive reaction on the screen test kit. A presumptive positive sample has typical colonies, observed on Rainbow Agar, and reacts specifically with 0157 antiserum. A sample is a confirmed positive sample for E.coli 0157:H7 or E. coli 0157:NM when the isolate is confirmed biochemically and serologically, and the presence of Shiga toxin(s) or Shiga toxin gene(s) is demonstrated.

Unless otherwise stated all measurements cited in this method have a tolerance of ± 2%.

5.1.2 Limits of Detection

This test has been shown to consistently detect less than 1 colony forming unit (cfu)/g in a 65 g sample.

5.2 Safety

E. coli OI57:H7/NM is a human pathogen with a low infectious dose. (Ingestion of 100 cells can cause disease.) The use of gloves and eye protection is mandatory and all work surfaces must be disinfected prior to and immediately after use. Laboratory personnel must abide by CDC guidelines for manipulating Biosafety Class II pathogens. A Class II laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. All available Material Safety Data Sheets (MSDS) should be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the materials should read all MSDS sheets.

5.3 Quality Control Practices

a. Rainbow® Agar plates have a shelf life of 2 weeks.

b. All media and E-Buffer must be pre-warmed to 18-35°C prior to use.

c. The recommended fluorescent strain of *E. coli* 0157:H7 must be used in this procedure to monitor for cross contamination. The protocol for the use of fluorescent strains of *E. coli* O157:H7 as positive controls follows:

Wild-type strains of *E. coli* O157:H7 transfonned with pGFP produce a green fluorescent protein. As a result of this transformation, fluorescent strains of *E. coli* 0157:H7 possess the unique property of expressing bright green fluorescence visible in the dark when illuminated by long-wave UV light. This property, which sets them apart from typical *E. coli* 0157:H7, makes them useful positive controls for analyses of meat samples for *E. coli* 01 57:H7/NM. At different steps in the procedure, both test samples and (fluorescent) positive controls can be tested for the bright green fluorescence as a Quality Control measure to make sure that positive sample isolates actually came from the test sample and not from accidental contamination by the positive control cultures.

Results of studies done at the FSIS Beltsville Microbial Pathogens Laboratory showed that these fluorescent cultures can be subjected to *E. coli* OI57:H7/NM isolation and identification procedures without losing their fluorescent properties. These strains retain their fluorescent properties when grown in SOB media with added ampicillin (SOB + A). These cultures must be transferred every 7 days to fresh SOB + A media, according to the protocol outlined below. The fluorescent colonies are ready to be used as positive controls on day 3 of the following protocol, and for the next 6 consecutive days without losing their fluorescent properties. If these cultures are not needed on a continuous basis, they can be stored at refrigeration temperatures on SOB + A agar plates in zip-lock bags or sealed with parafilm® for 1 month and then transferred, or started up again 2 days before needed. *Strict adherence* to the protocol described below is essential, in order to ensure that the fluorescent strains do not lose their ability to express green fluorescence.

i. Test the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97 or the currently designated control strain) on SOB + A agar plate for fluorescence by illuminating colonies under long-wave UV light in the dark.

ii. Select only fluorescing colonies and inoculate into 10 ml of SOB + A broth in a tube. Incubate at 35 ± 2°C overnight.

iii. Streak the culture from the SOB + A broth onto a SOB + A agar plate. Incubate at 35 ± 2°C overnight.

iv. Examine colonies on the plate for fluorescence. The fluorescent colonies are ready to be inoculated into modified EC broth + novobiocin (mEC+n) at this stage. These cultures on SOB + A agar plates can be stored refrigerated and be used as positive controls for 6 more days. Incubate the inoculated mEC+n positive control culture at 35 ± 2°C overnight, along with the test samples.

v. Continue analysis per Sections 5.5-5.7 and test the Blood Agar Plates of the fluorescent positive controls and any positive sample cultures for fluorescence.

5.4 Equipment, Materials, Media, Reagents and Test Kits

5.4.1 Equipment

a. Balance, sensitivity of 0.1 g
b. StomacherTM 400 or 3500 with appropriate sizes of sterile StomacherTM bags, with or without mesh. (Tekmar Co., Cincinnati, Ohio), or equivalent
c. Incubator, static 35 ± 2°C
d. Micropipettors to deliver 15-1000 µl with sterile disposable filtered micropipet tips
e. Mechanical Pipettor with 1.0 ml, 5.0 ml, 10.0 ml sterile pipettes
f. Inoculating loops, "hockey sticks" or spreaders, and needles
g. UV light (long-wave, e.g. VWR # 36553-124, or equivalent)

h. Filter unit, 0.2 µm, nylon, sterile
i. Infrared thermometer
j. LabQuake® Agitator (or equivalent) with clips to hold microcentrifuge tubes
k. Sterile disposable 12 x 75 mm polypropylene tubes (e.g. Fisher # 14-956-1B, or equivalent)
l. Microcentrifuge and sterile 1.5 ml microcentrifuge tubes
m. Sterile 50 ml conical tubes (e.g. Falcon® # 2070, or equivalent) or sterile bottles
n. Sterile 40 µm Cell Strainer (Falcon® # 2340, or equivalent)
o. MACS® Large Cell Separation Columns (Miltenyi Biotec # 422-02, or equivalent)
p. OctoMACS® Separation Magnet (Miltenyi Biotec # 421-09, or equivalent)
q. Multistand to support OctoMACS® Separation Magnet (Miltenyi Biotec # 423-03, or equivalent)
r. Tray, autoclavable, approximately 130 mm x 83 mm (e.g. VWR # 62663-222, or equivalent) for use with the OctoMACS®

5.4.2 Media, Reagents and Cultures

a. Modified EC broth with novobiocin (mEC+n) (or equivalent)
b. Rainbow® Agar 0157 (Biolog Inc., Hayward California, 94545) containing 10 mg/L novobiocin plus 0.8 mg/L potassium tellurite, or equivalent selective medium
c. Tryptic soy agar with 5% sheep blood
d. SOB + A Medium
e. E Buffer, approximately 7 ml per sample [Buffered Peptone Water, Bovine Albumin Sigma # 7906 (or equivalent), and Tween-20®, or equivalent]
f. Disinfectant (Lysol ® I. C., 2.0%, or equivalent)
g. Dynal® # 710.04 anti-*E. coli* 0157 antibody-coated paramagnetic beads (Dynal Inc., Lake Success, NY 11042), or equivalent
h. *E. coli* 0157:H7 strain 465-97 (positive control used throughout method)
i. *E. coli* ATCC strain 25922 (negative control for bead capture and screen tests)

5.4.3 Test Kits

a. The screening test for the detection of *E. coli* 0157:H7/NM should meet or exceed the following performance characteristics:

Sensitivity	≥98%
Specificity	≥90%
False Negative Rate	≤2%
False Positive Rate	≤10%

b. *E. coli* 0157:H7 latex agglutination test kit (RIM® *E. coli* 0157:H7 Latex Test Kit, REMEL, 12076 Santa Fe Drive, Lenexa, KS 66215, or equivalent)

c. Biochemical test kits and systems [Vitek® GNI and GNI Plus cards (bioMerieux Vitek, Inc., 595 Anglum Drive, Hazelwood, MO 63042-2395), or equivalent]

d. Shiga Toxin test kit [Premier® EHEC, cat. # 608096 (Meridian Diagnostics, Inc., 3471 River Hills Dr., Cincinnati, OH, 45244), or equivalent

5.5 Detection Procedure

a. Sample Preparation

i. Raw ground beef microbiological testing programs.
Randomly collect five 65 ± 2 g sub-samples (total of 325 ± 10 g) that are representative of the entire sample. Place each 65 ± 2 g sub-sample in a sterile Strainer StomacherTM bag. Add 585 ml mEC+n broth and pummel for two minutes in a StomacherTM.

ii. Cooked meat patties and semi-dry and dry fermented sausages. Randomly prepare five 65 ± 2 g sub-samples (total of 325 ± 10 g) that are representative of the entire sample. When appropriate, sample representative portions from both the outer surface (shell) and inner section (core) of RTE products, especially semi-dry and dry fermented sausages. Place each 65 ± 2 g sub-sample in a sterile Strainer StomacherTM bag. Add 585 ml mEC+n broth and pummel for two minutes in a StomacherTM.

iii. Outbreak-related samples. Randomly collect thirteen 25 ± 1 g sub-samples (total of 325 ± 13 g) that are representative of the entire sample. Place each 25 ± 1 g sub-sample in a sterile Strainer StomacherTM bag and add 225 ml of mEC+n broth. Pummel for 2 minutes in a StomacherTM.

b. Incubate all bags (static) with their contents for 20 to 24 h at 35 ± 2°C. Include a positive, negative, and uninoculated medium control for each group of samples tested. Use the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97) as a positive control and *E. coli* ATCC strain 25922 as the negative control.

c. From the enrichment cultures in the StomacherTM bags, perform the screening test for *E. coli* O157:H7/NM following the manufacturer's instructions. The enrichment culture may be analyzed immediately upon removal from the incubator without waiting for tempering to room temperature. To prevent clogging the pipette tip, be sure to collect the appropriate size sample from the enrichment culture outside the inner strainer bag.

d. Samples negative by the screening test can be reported as negative for *E. coli* 0157:H7/NM and discarded.

e. Samples positive by the screening test should be reported as potential positives. Begin isolation procedures from the enrichment culture in the StomacherTM bag.

5.6 Isolation Procedure

Note: Steps a.-1. may be performed in a sequence that is convenient to the laboratory personnel.

a. Prepare E Buffer by mixing 0.5 g Bovine Albumin and 50 µl Tween-20® into 100 ml Buffered Peptone Water (BPW). Filter sterilize (0.2 µm) and store at 2-8°C.

b. Remove Rainbow® Agar plates from 2-8°C storage, allowing 3 plates for each screen-positive culture and each control. Be sure that plates have no visible surface moisture at the time of use. If necessary, dry plates (e.g. for up to 30 minutes in a laminar flow hood with the lids removed) prior to use. Dried plates that are not used should be labeled "dried", placed in bags and returned to 2-8°C.

c. Remove a bottle of E Buffer from 2-8°C storage. Decant 7 ml of E Buffer for each culture and each control into a sterile tube or bottle and allow it to warm to at least 18°C. (Return the stock E Buffer to 2-8°C.)

d. For each positive control, negative control and screen-positive culture to be analyzed, order and label 50 ml conical centrifuge tubes so that the positive control is first, followed by the negative control, then all cultures. Maintain this order for subsequent steps.

e. For each positive control, negative control, and screen-positive culture, label two sterile 1.5 ml microcentrifuge tubes (for step g and step s), one 50 ml conical centrifuge tube (for step h.) and two 12 x 75 mm capped tubes (one for step p.). For each pair of 12 x 75 mm tubes, label one tube and add 0.9 ml E Buffer (for step q.).

f. Prepare the Dynal #710.04 *E. coli* 0157:H7 immunomagnetic bead suspension by following Table 1 below. Be sure to include the positive and negative controls in the total number of cultures. Use the bead suspension immediately (step g), or hold at 2-8°C. Return the stock vial of Dynal #710.04 *E. coli* 0157:H7 immunomagnetic beads to 2-8°C.

g. Vortex the bead solution briefly (2-3 seconds), then add 50 µl to a labeled microcentrifuge tube (from step e), one for each control and screen-positive culture. Use immediately or hold these tubes at 2-8°C.

h. Place a 40 µm Cell Strainer on a labeled 50-ml conical centrifuge tube (from step e.). Pipet 5± 1 ml of each control and enrichment culture into the respective Cell Strainer and collect at least 1.0 ml of filtrate.

i. Do not proceed with more than the number of tubes that the OctoMacs® magnet(s) will hold. Transfer 1.0 ml of a filtrate (step h.) to the corresponding microcentrifuge tube containing the immunomagnetic bead suspension (step g.) and place in the clips of the LabQuake® tube agitator. Rotate the tubes for 10-15 min at 18-30°C.

j. Attach the OctoMACS® Magnet to the Multistand.

k. Position a tray on the base of the Multistand so that it will collect the filtrate passing through the columns. Add approximately 300 ml of 2% Lysol® I. C. (or equivalent) disinfectant to cover the bottom of the tray.

l. Label and place the appropriate number of Large Cell Separation columns on the OctoMACS® Magnet. Insert columns from the front making sure the column tips do not touch any surfaces. Leave the plungers in the bags at this time to maintain sterility.

m. Transfer at least 0.5ml E Buffer to the top of each column and let the buffer run through.

n. Resuspend, then transfer each culture and control from step i. to its corresponding column.

o. After a culture or control has drained through, wash the column by applying 1.0 ml of E Buffer to each column and allow to drain. Repeat 3 more times for a total of 4 washes.

p. After the last wash has drained, remove the column from the OctoMACS® Magnet and insert the tip into an empty labeled 12 x 75 mm tube (from step e.).

Apply 1.0 ml of E Buffer to the column, and using the plunger supplied with the column, *immediately* flush out the beads into the tube. Use a smooth, steady motion to avoid splattering. Cap the tubes. Repeat this for each column. If the OctoMACS® magnet is to be used for a second set of cultures, it must be decontaminated as described in step u, below. Repeat steps j .- s. for the additional cultures.

q. Vortex the tubes from step p. briefly to resuspend the beads. Make a 1:10 dilution of each treated bead suspension by adding 0.1 ml of the bead suspension to a 12 x 75 mm labeled tube containing 0.9 ml E Buffer (from step e.).

r. Vortex briefly to maintain beads in suspension and plate 0.1 ml from each tube (from step p. and step q.) onto a labeled Rainbow® Agar plate. Use a hockey stick or spreader to spread plate the beads, being careful not to spread the beads against the edge of the plate.

s. Vortex the tubes containing undiluted beads (from step p.) and transfer to a labeled microfuge tube (from step e.) and centrifuge at least one minute using a bench-top microcentrifuge to concentrate the beads. Withdraw and discard the supernatant without disturbing the beads. Add 0.1 ml of E Buffer to the beads, resuspend the beads and transfer the beads to a labeled Rainbow® Agar plate. Spread plate the beads as described in step r.

t. As soon as there is no visible moisture on the agar surface, invert plates and incubate for 24-26 h at 35 ± 2°C.

u. Decontaminate the OctoMACS® Magnet by applying 2% Lysol® I. C. (or equivalent) disinfectant directly to the surface. After approximately ten minutes, rinse with deionized or tap water. Allow the unit to air-dry or use absorbent paper towels to dry the unit.

TABLE 1

# of Cultures	µ*l of Beads**	µ*l of E-Buffer*	*# of Cultures*	µ*l of Beads**	µ*l of E-Buffer*
1	15	135	26	145	1305
2	20	180	27	150	1350
3	25	225	28	155	1395
4	30	270	29	160	1440
5	35	315	30	165	1485
6	40	360	31	175	1575
7	45	405	32	180	1620
8	50	450	33	185	1665
9	55	495	34	190	1710
10	60	540	35	195	1755
11	65	585	36	200	1800
12	70	630	37	205	1845
13	75	675	38	210	1890
14	80	720	39	215	1935
15	85	765	40	220	1980
16	90	810	41	230	2070
17	95	855	42	235	2115
18	100	900	43	240	2160
19	105	945	44	245	2205
20	110	990	45	250	2250
21	120	1080	46	255	2295
22	125	1125	47	260	2340
23	130	1170	48	265	2385
24	135	1215	49	270	2430
25	140	1260	50	275	2475

*Dynal® anti-*E. coli* O157:H7 antibody-coated paramagnetic beads (vortex briefly before use)

5.7 Identification and Confirmation

a. After incubation, *E. coli* 0157:H7 colonies have black or grey coloration on Rainbow® Agar. When *E. coli* 0157:H7 colonies are surrounded by pink or magenta colonies, they may have a bluish hue. Mark colonies typical of *E. coli* 0157:H7 and perform latex agglutination assays for 0157, following manufacturer's instructions. Streak all latex positive colonies, up to a total of five from each sample (one per sub-sample, if possible) onto Blood Agar plates. Incubate Blood Agar plates for 16-24 h at 35 ± 2°C.

   Note: If no typical colonies are present, hold the original Rainbow® plates at 20- 24°C for an additional 6-24 h then re-examine for typical colonies.

b. After incubation, examine the Blood Agar plates for purity under visible light, and evidence of cross contamination with the positive control by using long wave UV light. Only the positive control culture, *E. coli* 0157:H7 strain 465-97, should fluoresce. If the Blood Agar plates appear pure and uncontaminated, perform the following confirmatory tests:

   i. Biochemical confirmation.
   Inoculate Vitek-GNI or GNI Plus cards or use an equivalent biochemical identification testing system. The cytochrome oxidase and gram stain tests are optional.

   ii. Serological confirmation.
   To confirm the absence or presence of 0157 and H7 antigens, use an *E. coli* 0157:H7 latex test agglutination kit (RIM® *E. coli* 0157:H7 Latex Test Kit, or equivalent). Use growth from the Blood Agar plate (from step b).

   iii. Shiga toxin/toxin genes confirmation.
   The presence of Shiga toxin(s) in a culture isolate should be confirmed by the use of a toxin assay, e.g., Meridian Premier® EHEC Kit, or equivalent. When Shiga toxin(s) is (are) not demonstrated, detection of one or more toxin genes by PCR should be used for confirmation. The positive control culture, *E. coli* 0157:H7, is toxin-negative.

c. If the isolate confirms as an *E. coli* OI57:H7, or *E. coli* O157:NM (or H-indeterminate) and the Shiga toxin(s) and/or one or more toxin genes are present, the sample will be treated as positive for *E. coli* 0157 :H7, and regulatory action will be taken. The cultures will also be tested by pulsed-field gel electrophoresis (PFGE) for potential epidemiological association.

5.8 Storage of Cultures

For storage requirements of the fluorescent *E. coli* O157:H7 strain (FSIS culture # EC 465-97 or the currently designated control strain), refer to Section 5.3.c. of this chapter.

Store other "working" *E. coli* stock cultures on nutrient agar slants. Transfer stocks monthly onto duplicate nutrient agar slants, incubate overnight at 35 ± 1°C, and then store them at 4-8°C. Use one of the slants as the working culture. Use the other slant for sub-culturing to reduce the opportunity for contamination. Cultures may be subcultured up to 5 times. After this period the culture must be re-confirmed biochemically or a new culture initiated.

For long term storage freeze cultures using cryo-beads i.e. CryostorTM or lyophilize.

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