Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments

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Ottawa (Ontario)
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November 17, 2010

MEAT HYGIENE DIRECTIVE

2010- 65

SUBJECT: Chapter 4

Changes to be made:

1. "Annex H: Meat inspection legend stamp order form" has been removed from Chapter 4 because it is now available as Annex A in MOP Chapter 1.
2. "Annex O: Policy on the Control of *E. coli* 057:H7 Contamination in Raw Beef Products" has been replaced with the most recent version that was updated and distributed as a Meat Hygiene Directive on August 7, 2009.

ENGLISH VERSION

Please remove Annex H of Chapter 4 from your Manual of Procedures.

Please replace Annex O of Chapter 4 with this new version in your Manual of Procedures.

FRENCH VERSION

Please remove Annex H of Chapter 4 from your Manual of Procedures.

Please replace Annex O of Chapter 4 with this new version in your Manual of Procedures.

Le 17 novembre 2010

DIRECTIVE DE L'HYGIENE DES VIANDES

2010- 65

OBJET : Chapitre 4

Changements à apporter :

1. « Annexe H : Formulaire de commande pour les étampes portant l'estampille » a été retiré du chapitre 4 à cause du fait il est maintenant accessible comme annexe A dans le chapitre 1 du MDM.
2. « Annexe O : Politique relative à la lutte contre la contamination des produits de bœuf crus par *E. coli* O157 :H7 » a été remplacée par la plus récente version qui a été mise en œuvre et distribuée comme une Directive de l'hygiène des viandes le 7 août 2009.

VERSION ANGLAISE

Veuillez supprimer l'annexe H de chapitre 4 de votre Manuel des méthodes.

Veuillez remplacer l'annexe 0 de chapitre 4 par cette nouvelle version dans votre Manuel des méthodes.

VERSION FRANÇAISE

Veuillez supprimer l'annexe H de chapitre 4 de votre Manuel des méthodes.

Veuillez remplacer l'annexe 0 de chapitre 4 par cette nouvelle version dans votre Manuel des méthodes.

Richard Arsenault
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Canada

Annex O

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

1. Effective date

This policy is effective on the date of publication.

2. Purpose

This policy is being updated for the following reasons:

- to provide clear guidance to industry and inspection staff on the measures required to control *E. coli* O157:H7 in raw beef products, and maintain equivalence with corresponding US policy; and
- to reflect the risk-based approach taken by the CFIA to address the risk posed by this pathogen.

All operators of registered establishments handling raw beef are required to proceed as follows:

- Reassess their HACCP system (prerequisite programs and CCPs) to ensure that it meets this policy's requirements. Please note that, if applicable, pertinent process controls must also be reassessed. Refer to items 4 and 5 of this policy for further details.
- For abattoirs: validate the pathogen reduction step(s) as per item 8 of this policy.

3. Definitions

In the context of this policy, the following definitions apply.

3.1 Accredited laboratory

An accredited laboratory, for the purposes of food testing to meet regulatory requirements, is one that is recognized by the Standards Council of Canada (SCC) as conforming to its Program for the Accreditation of Laboratories/Canada (PALCAN) requirements including the requirements of the Agriculture and Food Products Program Speciality Area.

3.2 Confirmed positive for *E. coli* O157:H7

A biochemically-identified *Escherichia coli* isolate that is serologically or genetically determined to be "O157" meets at least one of the following criteria:

1) positive for Shiga toxin (ST) production; and/or
2) positive for Shiga toxin gene(s) (*stx*); and/or
3) genetically determined to be "H7".

3.3 D value

A unit which expresses the lethality of a process. One "D" equals the destruction of 90% of the target organisms that may be present in the product. Hence, a process that achieves a 5 D reduction for *E. coli* O157:H7 is capable of destroying 10^5 of these organisms in the product, or achieving a 99.999% reduction in the number of any organisms potentially present.

3.4 *E. coli* O157:H7

E. coli O157:H7 is an enterohaemorrhagic, shiga toxin producing strain of *E. coli*. For the purpose of this policy, this pathogen is defined as follows:

- gives a positive test for detection of *E. coli* serotype O157 from an enrichment broth, and

- a pure isolate from the enrichment broth is confirmed with biochemical and serological tests as *E. coli* O157. Further, an accredited laboratory must confirm the pathogenicity of the isolate by a positive result on either of the following tests:
  - the production of Shiga toxin(s); and/or
  - presence of one or more of the Shiga toxin genes; and/or
  - serological and/or molecular test confirming the presence of the H7 antigen.

Note: All the results from the above listed options to confirm the pathogen must be found negative in order to report a **negative** final assessment report.

3.5 Full lethality treatment as applicable to beef products

In the context of this policy, a beef product is considered to have received a full lethality treatment when the manufacturing process has been scientifically validated to achieve a 5 D reduction of *E. coli* O157:H7.

3.6 Lot

When testing raw beef as per this policy's requirements, a lot is defined as comprising all cartons, packages or containers of raw beef either:

- packed on a given packing line and based on Sanitation Standard Operating Procedures; or
- determined by the operator when implementing a statistically based sampling program (robust testing or alternate sampling protocol accepted by the CFIA).

A lot may be defined either in time or space but cannot exceed a single day's production.

3.7 Presumptive Positive for *E. coli* O157:H7

A sample that causes a positive reaction with a CFIA recognized screening test (see Appendix 2 of this policy).

3.8 Raw beef

For the purpose of this policy, the term raw beef includes beef, veal as well as their hearts, head meat, cheek meat, oesophagus, etc. (i.e. striated muscle). Raw beef includes intact beef products, non-intact beef products and comminuted beef products.

Note: Beef tails and tongues are excluded from the raw beef product definition since they are customarily fully cooked and have not been linked to human illnesses.

3.8.1 Intact raw beef

Intact raw beef is a piece of meat whose internal structure has not been modified. This category includes: dressed carcasses in whole/half or quarter format, primal and sub-primal cuts, trimmings removed from the aforementioned parts, head meat, cheek meat, diaphragm, and intercostal muscle.

3.8.2 Non-intact raw beef

Non intact raw beef is beef that has been either:

- tenderized;
- injected;
- submitted to a process that incorporates a solution (e.g.: massaging, tumbling); or
- diced.

Comminuted beef

This includes ground/comminuted beef, finely textured beef and mechanically separated beef.

3.9 Robust testing of beef trim or raw beef material that is used in the production of ground beef

A minimum of 60 sub-samples must be examined per lot.

A lot cannot exceed 5 combos and cannot weigh more than approximately 4,500 kg. An alternate unit to a combo may be defined and used by the operator (e.g., a pallet of boxes or a tote, buggy, vat, tub, etc.), provided the weight of the lot does not exceed approximately 4,500 kg and does not exceed an entire day's production.

All combos/units must be equally represented in the sample. For example, a minimum of 12 individual pieces would be taken from each combo of a 5 combo lot. For alternate units, a minimum of 60 equally distributed pieces must be collected across the lot (e.g. a 10 vat lot of bench trim could be sampled by collecting 6 pieces per vat, a 5 pallet lot could be sampled by collecting 12 pieces per pallet, etc.).

A minimum of 325 g of material from each lot shall be collected and submitted for testing. At least 65 g of material (12 pieces weighing 5 or 6 g each) would be collected from each combo in a 5-combo lot. For alternate units the amount of material collected from each unit would depend on the number of units in the lot but would still be made up of 5 or 6 g pieces collected equally from each unit, adding up to a minimum total of 325 g for the lot. The material collected for testing should represent the outside surface of the product (e.g. carcass surface for sampled trim, exposed surfaces of the heart muscle, external aspect of the diaphragm muscle, etc.), i.e. it must not be taken from inner meat tissue unless the normal production process has left only inner tissue to sample.

Notes:

1. In the N-60 sampling procedure, 60 represents the minimum number of sub-samples required, regardless of the size (number of combos/units) and weight of the lot. Whether the lot weighs 4,500 kg, 2,000 kg or 100 kg, 60 sub-samples must be collected from the lot.

2. Alternate parameters may be used to define robust testing provided they have been evaluated by the CFIA (the Meat Programs Division and Food Safety Division) and provide an equivalent or increased level of confidence.

4. Risk posed by *E. coli* O157:H7

Operators are required to keep their HACCP system up-to date so that all applicable regulatory and policy requirements are met. In light of the updating of policy requirements, operators of all establishments that produce or receive raw beef products are required to **reassess their HACCP system** to ensure that it meets this policy's requirements. For the purpose of this policy, the term HACCP system encompasses all process controls that need to be used by the operator to control *E. coli* O157:H7.

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. One should keep in mind that *E. coli* O157:H7 is an unacceptable contaminant in beef and that contaminated products are subject to recall.

As part of the reassessment, the following information must be included in the HACCP system:

- FSEP forms (or equivalent) on **product identification** and **intended use** are to be reviewed for completeness and accuracy with regards to the *E. coli* O157:H7 hazard and controls;

- the *E. coli* O157:H7 hazard shall be clearly identified on the FSEP form # 5 (or equivalent). This hazard must be passed through the decision tree (FSEP form # 8 or equivalent);

- the operator has to determine how the hazard will be addressed:
  - through CCP(s) at the establishment during production;
  - when receiving raw beef products or

- through other validated measures that will prevent distribution of potentially contaminated products.

For abattoirs, particular attention must be paid to airflow, as well as employee movement between the clean and the relatively less clean areas of the processing areas as these factors have recently been strongly associated with the microbiological contamination levels of the carcasses. Segregation of incompatible activities, product flow, equipment sanitation as well as employee training (Good Manufacturing Practices [GMPs], hygienic handling, dressing procedures etc.) should also be evaluated. Finally, as adequate refrigeration plays an important role in preventing the multiplication of *E. coli* O157:H7, operators should confirm that they have sufficient cooling capacity to handle their volume of production while meeting the carcass cooling guidelines found in the Meat Hygiene Manual of Procedures, Chapter 4.

The operator must ensure that all pertinent aspects of his HACCP system have been updated as required.

5. Control measures/interventions

Operators shall ensure that their HACCP system has been validated and is effective, such that the level of *E. coli* O157:H7 in raw beef products distributed outside of federally registered establishments is below the detectable level (i.e., no *E. coli* O157:H7 detected in a sample when tested with one of the approved methodologies [please refer to Appendix 2 of this policy for the relevant information]). 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:

5.1 Abattoirs

Cattle are the primary source of *E. coli* O157:H7 that infects humans. The shedding of *E. coli* O157:H7 in cattle feces is intermittent and increases during summer and fall season. Contamination of beef carcasses with *E. coli* O157:H7 occurs during slaughtering and dressing procedures, especially during the de-hiding process.

Subsequent use of any meat components derived from a contaminated carcass may find its way into raw ground beef, which is considered the greatest risk in causing human illnesses. In the case of a slaughter establishment, as a result of the reassessment of the operator's HACCP system, the operator is expected to:

5.1.1 All abattoirs

- Use one or more interventions, such as steam pasteurizers, organic acid sprays, etc., validated according to this policy (please refer to item 8 of this policy), at the time of slaughter to reduce the *E. coli* O157:H7 contamination to below detectable level; cover this (these) intervention(s) with a CCP.
- Control air contamination, airflow (positive pressure) and employee movements between clean and relatively less clean areas of the processing areas.
- The storage and transportation prerequisite program must indicate that conditions under which the carcasses were kept were satisfactory. This includes, but is not limited to, storage temperature.
- Implement a verification step to ensure the effectiveness of the interventions covered by the CCP. This procedure must include testing of the carcasses for the pathogen or a surrogate organism, as described in item 9.1 c of this policy, in the slaughter establishment or at receiving in an affiliated receiving establishment. In the latter case, the operator of the abattoir must first present the integrated plan to the CFIA, and the CFIA must accept the plan for this option to be allowed.

5.1.2 Abattoirs with boning/cutting operations

For abattoirs which also have boning/cutting operations, **in addition to the requirements stated under 5.1.1 above**, the following requirements apply to carcasses being processed as well as to product obtained after the cutting/boning operations:

- Carcasses must have been subject to the pathogen reduction intervention(s) (validated CCP as per 5.1.1) used at the establishment; pertinent HACCP records must indicate that the CCP was under control;
- The storage prerequisite program must indicate that conditions under which the carcasses were kept until being boned/cut were satisfactory. This includes, but is not limited to, storage temperature as well as cross-contamination potential;
- Conditions under which the carcasses were further processed (cutting/boning) were satisfactory and met the HACCP system specifications;
- If any of the raw beef products obtained from the boning/cutting processes tests positive for *E. coli* O157:H7 (whether when the test is performed at the establishment or when product is received by a customer), the investigation must also include the evaluation of all the parameters of the slaughter process that have an impact on the presence of *E. coli* O157:H7.

5.2 Receiving establishment

After the reassessment of their HACCP system, the operator receiving raw beef must choose either one of the following options:

- Implement purchase specifications (please refer to item 6 of this policy) and determine that the control provided by either the Receiving CCP or the Transportation, Receiving & Storage Prerequisite Program (B 2.1.3) receiving step is adequate to ensure that these purchase specifications are met. These purchase specifications must require that all suppliers have one or more validated CCPs in their production of raw beef shipped to the receiving establishment and that their controls are implemented and effective;
- Determine that a 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 or subjected to another accepted full lethality treatment).

5.3 Mandated testing of beef trim and other raw beef precursor materials that are used for the production of ground beef

Alternate parameters may be used for robust testing provided they have been evaluated by the CFIA (the Meat Programs Division and Food Safety Division) and demonstrate an equivalent or increased level of confidence.

All raw beef products intended for use in the manufacture of ground beef (ground beef precursor materials) are subject to the testing requirements described in item 5.3 of the present policy prior to its use in the manufacture of ground beef. Unless demonstrated otherwise by the operators (through documented evidence and controls), the raw ground beef precursors identified below will be considered as potential input material for the production of ground beef.

Because they are commonly used in ground beef production, products subject to **mandated testing** include trim and bench trim (trim derived from primal and sub-primal cuts), head meat, cheek meat, weasand meat, hearts, finely textured beef. As such, operators who **generate** these raw ground beef precursors will be required to test these products in accordance to item 5.3 of this policy.

Also, if other less common raw beef components such as primal or sub-primal cuts are destined for use in the manufacture of ground beef products (e.g. sirloin burger), the products must be tested prior to distribution at the frequency indicated below.

The testing frequency has been determined according to the volume of production, which has a direct impact on the level of risk related to consumer exposure. The tests used must satisfy the conditions set out in Appendix 2 of this policy document.

Operators must therefore establish their yearly production volume (in kg) for the trim, as well as for any of the other raw ground beef precursor materials they produce, or a combination of them that may be used in the production of ground beef. When only part of the production of a given product or a combination of them will be used for the production of ground beef, operators only need to test the product that will be used in the production of ground beef. This must be adequately documented in the operator's HACCP plan. For instance, records indicate that 30% of the hearts or a combination of hearts and cheek meat produced yearly are used for the production of ground beef. This means that the hearts or the combination of hearts and cheek meat making up that 30% of total yearly production must be tested at the applicable frequency.

When the use of the ground beef precursor material can not be verified by the CFIA (e.g. product is sold outside of the federally registered sector), it will be considered as a potential input for the production of ground beef and **must** be included in the production that falls under the testing requirement. Exemptions from mandatory testing will only be considered by the CFIA if there are suitable controls (documented evidence in HACCP) in place to ensure that these components are not used to make ground beef, and provided that the receiving facility is a federally registered establishment.

Operators must keep this information current and accurate. For instance, any increase in the production must be evaluated to determine if it impacts the testing frequency of a given product.

When receiving raw materials used in the manufacture of raw ground beef, the operator has verified that the raw materials have all been tested by the supplier as follows:

5.3.1 Production of 25,000 kg or less per year of a given product

Considering the small volume of product this represents, operators need to test this product three (3) times per year as per the N=60 sampling procedure. Seasonal variation, i.e. where the prevalence of *E. coli* 0157:H7 is the highest (from summer to fall), should be considered when determining when to test in the year.

5.3.2 Production of over 25,000 kg per year of a given product

These operators must implement a robust testing protocol, as defined in this policy, for any product that falls under this category.

5.4 Raw beef used to manufacture fully cooked products or used in a process that includes an accepted full lethality treatment

As a result of the reassessment of its HACCP system, the operator may determine 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 subjected on site to a full lethality process (e.g., cooked to achieve a 5D reduction) or shipped directly to another federally registered establishment where similar steps are taken to control the hazard.

Where this occurs, the operator must provide details on the control measures in place to ensure that the product receives the full lethality process and that it is not diverted elsewhere (i.e., how inventory is managed, if boxes have special markings [e.g., "For Cooking"/"For Full Lethality Treatment"]). The measures need to be incorporated and evaluated for compliance and effectiveness within the operator's HACCP system.

In the case of product sent for a lethality treatment after it has been tested and found positive for *E. coli* O157:H7, or product that the operator has chosen to treat as positive for the pathogen based on screening results, refer to item 13.1.1 of this policy.

5.5 Production of non-intact raw beef products

For products which will be subjected to **dicing, massaging/tumbling, mechanical tenderization, needling or injection**, the equipment used may translocate bacteria from the surface of a contaminated cut of meat to its interior, as well as cross contaminate subsequent portions processed by the same machine.

The non-intact raw beef products do not present the same level of risk as the raw ground beef precursors. The certificate of analysis or verification testing is not mandatory for such products. However, the operators producing non-intact raw beef products have to implement control measures according to "industry best practices" which may include review and reassessment of sanitation process, equipment cleaning and other activities such as proper labelling and cooking instructions. If such products or beef trims are found to be associated with a positive *E. coli* O157:H7 result the operator must justify the deviation and take appropriate corrective and preventative measures.

5.6 Raw intact primal and sub-primal cuts

It should be noted that intact primal and sub-primal cuts used for purposes other than the manufacture of ground beef do not pose the same level of risk as ground beef. In contrast to ground beef, the interior of these 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. An establishment producing and distributing pre-packaged intact steaks may conclude it does not need to change its HACCP system for these products.

Trim generated during the manufacture of primal and sub-primal cuts must be subjected to controls to ensure that the end products into which they will be used do not contain detectable levels of *E. coli* O157:H7. As trim is commonly used for the production of ground beef, they must be tested at the frequency prescribed under item 5.3 of this policy. Alternately, this trim may be used for the production of a product that will be cooked or subjected to a full lethality treatment in a federally registered establishment and under a HACCP system that addresses the potential *E. coli* O157:H7 contamination hazard.

Should any part of beef cuts such as primal and sub primal cuts, including boneless chucks that are generally not destined for the production of ground beef, be used by an operator for that purpose, it **must** be subjected to robust testing applicable to raw beef trims and other raw ground beef precursors. This testing may be done by the operator producing these products (at their buyer's request) as per items 3.9 and 5.3 of this policy, or by the operator receiving these products as per items 3.9 and 9.2 c of this policy.

6. Purchase specifications

The following **purchase specifications** must be recognized and filed accordingly by receiving operators in their HACCP system:

(1) A **letter of guarantee** from supplying companies must be on file identifying the validated intervention(s) (CCP) as well as other measures used to reduce, prevent or eliminate the hazard associated with *E. coli* O157:H7 for all beef products. The letter must be dated and signed by the operator, or a designated person, of the supplying establishment. For establishment shipping product pending result, the letter must include a statement to the effect that when the supplier obtains an unsatisfactory result, the operator(s) of the establishment(s) which have received implicated product will be notified as well as the supplier's local CFIA inspection staff.

Notes:

1. Once notified, the operator(s) of the receiving establishment(s) must in their turn inform their respective CFIA Inspector in Charge.
2. A certificate of analysis, although providing an increased level of confidence, cannot be used in lieu of 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.

2) As a **verification step for the receiving CCP or Prerequisite Program B 2.1.3**, the receiving operator must verify that the supplier is applying effective measures for the control of *E. coli* O157:H7 **in raw beef trim and other ground beef precursor materials used in the manufacture of ground beef, and is doing all testing required by item 5.3 of this policy**.

The receiving operator may achieve this by specifying the following requirements within purchase specifications for each of their suppliers:

2.1) **For suppliers producing more than 25,000 kg per year of raw beef trim and ground meat components (per category):**

- The supplier performs robust testing on each production lot of raw beef trim and ground meat components as per item 5.3 of this document.
- The supplier provides a certificate of analysis (COA) for each lot of raw beef trim and ground meat precursors sent to the receiving establishment. The COA must indicate results of testing for the specific lot being sent. The result of the test must be negative (*E. coli* O157:H7 not detected).
- Upon approval by the CFIA, an alternate method which is able to provide the same level of control as use of the certificates of analysis may be considered.
- The supplier is subjected to third-party audits and can demonstrate through the audit reports that the above requirements (section 2.1) are all being implemented in a suitable manner. Third-party audits must be conducted by a firm, organization or individual who has a proven track record in performing this type of review. On an exceptional basis and with the agreement of the supplying establishment, an audit may be conducted by an employee of the receiving establishment. The CFIA reserves the right to reject an auditor who fails to suitably demonstrate that he or she meets the above criteria or who refuses to provide reports to the CFIA upon request.

Note: (For suppliers producing more than 25,000 kg per year)

If all of the above requirements are met, the receiving establishment would not need to perform verification testing of received lots of raw beef trim or ground beef components.

2.2) **For suppliers producing 25 000 kg or less per year of raw beef trim and ground meat components (per category):** a letter to that effect is provided by the supplier and kept on file by the purchaser. The supplier also confirms on a yearly basis that the requested tests have been performed.

7. Cross-contamination potential

In the case of an operator 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 to ensure that raw beef products received for cooking or other full lethality process are not used in the production of finished raw ground beef products and that cross-contamination is prevented.

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 ground beef product manufacturing and not for those received for further cooking or other full lethality process. For audit purposes, these segregation procedures would be audited under the appropriate Prerequisite Program.

8. Validation of pathogen reduction step(s) - Abattoirs

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 (e.g., intervention "Y" should result in a 2.0 log reduction considering parameters defined under experimental conditions [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 operator 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 four (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.** Please see Appendix 1 for guidance on sample size, **required number** of samples (Table 1) and statistical analysis, and refer to Annex T of the United-States section of Chapter 11 of the Meat Hygiene Manual of Procedures for the complete information about the testing procedure.

Step 3 The sum of the log reductions from all interventions (CCPs) must result in a final product that is below the 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 (1) month** of production as reflected in Table 2, Appendix 1. Based on the current prevalence of *E. coli* O157:H7 in raw beef products, sampling plans used for validation of controls must use a 0.1% expected prevalence. Sampling of carcasses should be conducted in accordance with Annex T of the United States section of Chapter 11 of the Meat Hygiene Manual of Procedures for the complete information about the testing procedure.

Notes:

- If the lot size (i.e., number of animals slaughtered) falls between two values appearing in column 1 for Tables 1 and 2, the highest number should be selected in order to determine the sample size.

- It is the prerogative of 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. Laboratory results (laboratory tests certificates) shall indicate which method has been used to analyze 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 (i.e. that are below detection level for *E. coli* O157:H7). Validation has to be conducted again when the operations have changed substantively and/or pathogen intervention(s) has (have) been added or modified in a novel manner. The plant management and VIC/IIC may consult with the area program specialist regarding the need to revalidate a pathogen reduction intervention.

9. Verification activities and testing

9.1 Abattoirs (with or without boning/cutting operations)

Pathogen reduction steps are expected to become part of a CCP when passed through the decision tree (FSEP form # 8). Verification must therefore 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; and
c. an appropriate level of random product testing is done on a routine basis for *E. coli* O157:H7 or a surrogate organism. For the purpose of this testing, a lot may be defined as one carcass that is randomly selected for testing. The carcass, as well as one carcass preceding and following the tested carcass, should be held pending the receipt of results. In the event that the result for the tested carcass is positive, all three carcasses are considered as contaminated (unless tested as per item 10 of this policy) by the pathogen and must be subjected to a full lethality treatment or denatured and condemned.

   When operators decide to test for a surrogate organism, they will have to meet the following conditions:

   - Justify the choice of the surrogate organism used for that purpose.
   - Indicate what level of that organism will be considered as a non-compliance (critical limits) and provide the rationale for that decision.
   - Include in the HACCP system the corrective actions that will be implemented when the concentration of the surrogate organism is above the critical limit.
   - This option may be used after the CFIA has reviewed and accepted the operator's proposal for the use of this surrogate organism.

Please refer to Annex T of the United States section of Chapter 11 of the Meat Hygiene Manual of Procedures for complete information about the testing procedure. Acceptable laboratory methodologies are listed under Appendix 2 of this policy. The following minimum testing frequencies, based on the average production volume per month, have been established:

Establishment category	Average production volume per month	Frequency of sampling*
High volume	More than 5000 heads	One carcass every month
Medium volume	500 to 5000 heads	One carcass every other month
Small volume	Less than 500 heads	One carcass every three months

* It is the operator's responsibility to implement these activities at a frequency which will ensure that finished product will meet applicable requirements.

Note:

Abattoir operators that can provide test results from downstream *E. coli* O157:H7 carcass testing performed by affiliated operators at receiving of their carcasses may be exempted from carcass testing in their own establishment as indicated under item 5.1.1 of this policy.

9.2 Receiving establishments

Operators receiving raw beef products must ensure that the products received meet their purchase specifications either by a Receiving CCP or the Transportation, Receiving & Storage Prerequisite Program (B 2.1.3). Verification must therefore be performed. 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. the operator has verified that the raw materials used in the manufacture of raw ground beef have all been tested as required under item 5.3 and met all the requirements under purchase specifications in item 6.2 of this policy. In such cases, verification testing of raw beef products at receiving is not required. When receiving untested raw beef products that will be used for the production of ground beef, the receiving establishment must conduct verification testing at receiving. The following requirements apply:
    - product tested must be from a single supplier;
    - no co-mingling of lots can be done (each lot must be sampled on its own);
    - product must be tested under robust testing (or an alternate protocol that is equivalent or better); and
    - presumptive positives must be confirmed.
d. auditing of suppliers (optional) may also be performed as a verification activity.

It is the operator's responsibility to implement these activities at a frequency which will ensure that finished product will meet applicable requirements.

10. Lot considerations

The following guidelines should be used to define a lot for the purpose of sampling carcasses, trimmings, manufacturing beef or ground beef for *E.coli* 0157:H7:

- 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. It is strongly recommended that the lot, and any raw product manufactured from the lot, be detained pending receipt of laboratory results. 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.

- For trimmings, as well as other raw beef material that may be used in the production of ground beef, the CFIA would recognize the operator's definition of the sampled lot, provided the operator has implemented a robust sampling plan as per item 5.3 of this policy.

- For the purpose of carcass testing, it has been accepted by both Health Canada and the CFIA that the tested carcass represents a lot on its own. However, to address cross-contamination potential when a carcass is found positive for *E. coli* O157:H7, the tested carcass as well as the one carcass preceding and following the tested one will be considered as positive product, unless proven negative by a laboratory analysis using a specific methodology listed in Appendix 2.

- For other raw beef products, if no satisfactory scientific basis is provided by the operator for lot definition, the **default lot considered** by the CFIA will be from clean-up and sanitation to the next clean-up and sanitation.

- It should be noted that if an operator has a validated HACCP system and regularly tests specific lots of product that 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. Transit of raw product within the federal system

When an establishment receiving raw beef is in turn supplying raw beef products to another establishment, the following guidelines apply:

- Both operators must have purchase specifications to prevent *E. coli* O157:H7 from entering their facilities, which could be based on those described in item 6 of this policy, as part of their verification activities. In this case, the letter of guarantee provided by the establishment that receives and ships raw beef should state that the operator has a control in place to address the

risk associated with *E. coli* O157:H7 at receiving (CCP or pre-requisite program), and has letters of guarantee from all of their suppliers on file.

- In addition to purchase specifications addressing *E. coli* O157:H7 referred in 5.2, receiving establishments must ensure that measures are in place to prevent *E. coli* O157:H7 growth or contamination after product is received.

For additional information on letters of guarantee, please refer to item 6 of this policy.

12. Shipping of tested product pending receipt of results

Operators must maintain control over tested products until results are obtained. This will prevent tested products of unknown status from being offered to the public until they have been found satisfactory.

Operators who elect to ship tested product prior to being informed of the laboratory results must therefore inform the recipient that the product cannot be used before they are notified that the results were satisfactory. When a product has been distributed prior to its testing results being known and the test results indicate a potential or confirmed *E. coli* O157:H7 result, the CFIA must be immediately notified and the incident treated as a potential recall situation. The CFIA strongly advises against this procedure.

When tested product is shipped pending reception of results, the following conditions apply:

a) The **shipping** establishment must:

- maintain complete records identifying the type of product, as well as the quantity, being shipped
- identify the product in an appropriate way
- control product while it is in transit - the use of company seals is mandatory; and
- get confirmation from the receiver that the product (specifying the type and quantity) was received (note: this should be stated in the operator's protocol).

b) The **receiving** establishment must:

- maintain complete and accurate records of all products received under these conditions - this information must be captured in its HACCP files; and
- keep the product segregated until the laboratory results are obtained.

Notes:

- The above requirements also apply to tested product shipped outside of the federally registered system pending receipt of results.
- Operators cannot export tested raw beef products pending receipt of results.

13. Positive results for *E. coli* O157:H7

Unless otherwise specified within the policy (e.g. see item 9.2 c), presumptive positive results *may* be considered as positive results by the operator. When this is the case, the measures that apply are the same as if the laboratory result had been a confirmed positive. When this presumptive positive result impacts on another establishment (e.g. product tested at the receiving step was a presumptive positive), the operator performing the test must have a prior agreement with the supplier as to whether a presumptive positive is accepted as a positive result, OR a complete laboratory confirmation will be performed to determine either a positive or negative result (please refer to Appendix 2 of this policy). All registered establishments who purchase raw beef must document prior agreements in their HACCP systems. This ensures that product disposition and follow-up for both the supplier establishment and purchasing establishment can proceed expediently when a presumptive positive result is obtained.

When obtaining positive results for *E. coli* O157:H7, whether confirmed or considered as positive, the operator must:

- dispose of the positive product appropriately, as per item 13.1 of this policy; and
- take immediate action, as per item 13.2 of this policy.

13.1 Product disposition

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

1. Process the product into a fully cooked finished product within the federally registered sector. If done in a different establishment, it must be transferred under company seal and the adulterated product must be sent directly to an establishment that provides the heat treatment to the product; appropriate records must be kept by all involved operators to ensure complete control over the product until the risk has been addressed. OR

2. Denature and condemn the product under the direct supervision of the CFIA. OR

3. In the case of a product received from another registered establishment, the operator may reject the product and, providing the supplier has agreed in advance, return the product to the supplier under company seal for appropriate disposition. Records must be kept by both operators to ensure that the positive product is adequately controlled until it is subjected to either one of the two options described above.

Whichever option is selected, the traceability component must be covered in detail in the operator's HACCP system.

Note:

Operators may ask the CFIA to store positive product prior to submitting it to the lethality treatment (cooking). Any such request must be presented to the Area Program Specialist for evaluation. If accepted, the HACCP systems of both the shipping establishment and the storage establishment must address this situation. Appropriate controls must be in place and monitored (including but not limited to product inventory, segregation procedures, seals used for transit, etc.).

13.1.1 Product salvage - Cooking

In all cases where product is salvaged, as per the above first option, the following requirements apply:

- The cooking process must be approved by the area Program Specialist (meat processing) before being used;
- The operator must maintain a complete and up to date inventory of all product being salvaged because it was found positive for *E. coli* O157:H7 or because the operator has chosen to treat it as positive for *E. coli* O157:H7. The inventory shall include the following information: initial lot numbers and test results, type of product, weights, time/date of cooking, lot numbers of finished products, weights, and finished product test results; and
- All lots of cooked products made from components that come from a lot with a ***presumptive*** or ***confirmed*** *E. coli* O157:H7 positive product must be tested and found negative for the pathogen prior to being released for sale (one sample of 5 x 65 g must be analyzed for each such cooked lot of product made from raw beef that did not have a satisfactory laboratory result).

When positive product is **shipped** to another federally registered establishment for cooking, the following conditions apply:

a) The **shipping** establishment must:

   - Maintain complete records identifying the type of product, as well as the quantity, being shipped for cooking.

- Label the product with “For cooking only” (stamp or sticker). If the product is being stored in an off-site registered storage at the time that the *E. coli* O157:H7 test results are known, the product can be labelled at the storage facility, with the consent of the CFIA inspector.
- Control product while it is in transit. The use of company seals is mandatory.
- Get confirmation from the receiving establishment that the product (specifying the type and quantity) was received. (Note: this should be stated in the operator’s protocol.)

b) The **receiving** establishment must:

- Confirm the reception of positive product with the shipping establishment.
- Maintain complete and accurate records of all positive products received for cooking process.
- Meet the three requirements applicable to this procedure (approved cooking process, testing end product for the pathogen and inventory of product).

13.2 Follow-up actions - Operators

Any raw beef product that is positive or presumptive positive (positive screening test result) for *E. coli* O157:H7 is considered adulterated by the CFIA. This is why the operator must inform the CFIA of this situation whenever it arises. For auditing purposes, the information must be presented to the CFIA in written form.

13.2.1 Positive results obtained from products produced at the establishment

Operators using a robust testing protocol as a process control will very likely get more positive results than operators who test only for verification purposes. The CFIA still expects these operators to comply with items 13.1 and 13.2.1 of this policy. However, the impact of any positive result must be part of the trend analysis done on all laboratory results. Any potential link between positive results must be evaluated. When the number of positives exceeds what is expected (based on the prevalence of the pathogen and the level of testing applied), the operator should conclude that the **measures** to control the risk posed by *E. coli* O157:H7 must be increased (better or additional pathogen reduction steps).

The operator must take these 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 inspector in charge (IIC) /veterinarian in charge (VIC) of the establishment, who will in turn notify their Inspection Manager and the Area Program Specialist. The operator must also take the following steps:

1. Ensure that any affected product is under control.
2. Investigate the cause of the deviation by evaluating, as appropriate:
   a. all applicable HACCP controls;
   b. Sanitation Procedures (Prerequisite Programs);
   c. GMPs; and
   d. any other pertinent documentation and procedures

   Notes:

   i. This also includes beef **slaughter** activities, whether these take place on site or at another registered establishment.
   ii. While each positive must be investigated in an effort to identify the probable cause and to guide corrective measures, the scope and depth of an investigation may be adjusted according to current circumstances, taking into account such factors as the frequency and number of positives being found (trend analysis), and the type(s) of product(s) affected.

3. Apply a corrective action to eliminate the deviation cause.
4. Ensure that the corrective action has brought the CCP and/or Prerequisite program(s) under control.
5. Perform a food safety assessment on the affected product and determine if other products were implicated. Determine the appropriate disposition.

6. Implement preventative measures to prevent recurrence of the deviations. Serious consideration should be given to increase the lethality of the pathogen reduction steps used in the HACCP system.
7. Verify the effectiveness of the preventative measures. When the pathogen reduction step has been substantively modified to increase its efficacy, the intervention 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 item 8 of this policy. Validation of pathogen reduction step(s)). The plant management and IIC/VIC may consult with the area program specialist regarding the need to revalidate a pathogen reduction intervention.
8. For each corrective action and preventative measure, the designated employee must specify on the record: a target date for completion of corrective actions and preventative measures; the actual completion date for these corrective actions and preventative measures. Each entry includes the date, and is signed or initialled by the establishment employee making the entry.
9. Provide the inspector with the operator's action with regards to the adulterated product explaining how the product will be handled, controlled, brought back into compliance or disposed of (denatured and condemned).

If no contamination source can be identified a report indicating this should be produced and made available to the CFIA, as well as the rationale used to reach that conclusion. The report should include:

- documents reviewed;
- procedure used to review;
- dates reviewed;
- evaluation of the impact of this positive result on their own testing results for this type of product; and
- appropriate signatures of reviewer(s).

In all cases: **The inspector evaluates the investigation done by the operator in collaboration with the designated area Program Specialist, Complex Supervisor and Regional Veterinary Officer. When the investigation's conclusion or the corrective actions taken are judged inadequate, a Corrective Action Request (CAR) is issued under Compliance Verification System task 7101.**

13.2.2 Positive results obtained as a result of verification testing done on products received from a supplier

In the case where a positive result is obtained as a result of verification testing done on products received from a supplier, the following requirements apply:

1. The operator having performed the test must immediately notify the Inspector in Charge and the supplying establishment of the unsatisfactory results. The supplying operator will then inform the IIC/VIC at their establishment of the unsatisfactory result. The IIC/VIC of the supplying establishment will inform their own Inspection Manager and Area Program Specialist of the situation.
2. The receiving operator must dispose of affected product as per one of the three options presented under item 13.1 of this policy.
3. The supplying establishment must treat this notification as evidence that their HACCP system may have been ineffective in producing a product with *E. coli* O157:H7 below the detectable level and must investigate the situation accordingly and take immediate follow-up actions as per item 13.2.1 above.

The Area Program Specialist will ensure that the necessary details are communicated to any other CFIA Area Staff who may need the information.

13.3 Follow-up actions – CFIA

Any raw beef product that is positive or presumptive positive (positive screening test result) for *E. coli* O157:H7 is considered adulterated by the CFIA. That is why the operator must inform the CFIA of this

situation whenever it arises. For auditing purposes, the information must be presented to the CFIA in the written form.

As per section 20(2) of the *Meat Inspection Regulations, 1990* (MIR), the inspector will hold (held tag CFIA/ACIA 0093) the product until it is made to conform to those standards by the operator. The inspector will keep on file the appropriate information as per the Meat Hygiene Manual of Procedures section 4.1.1(4), Use of held tags CFIA/ACIA 0093 under control mechanisms.

The inspector must also indicate at the back of the held tag CFIA/ACIA 0093 the sections under which the detention is taken, which are section 20.(2), and section 130 of the MIR for the necessity to obtain from the inspector the authorization to remove an official tag or to handle or use this meat product.

The inspector must be provided with the operator's risk management plan, including the action plan, with regards to the adulterated product. Files must be kept with all the needed information (e.g. product involved, quantity, lot identification, link to the laboratory result).

If adulterated product is sent to another federally registered establishment for cooking, the HACCP plan of this (these) other establishment(s) must also address the needed controls. In such cases, the CFIA inspector of the other establishment must be notified on each occasion.

As per section 130 of the MIR, the inspector must either remove the held tag in person or authorize its removal. This must also be documented as it pertains to this specific issue in both the operator’s HACCP plan and the inspector’s files.

Notes:

- If the product was imported, the Area Program Specialist will immediately notify in writing the National Specialist, Import Programs, Meat Programs Division, in Ottawa. The National Specialist, Import Programs will notify the authorities of the exporting country for a follow-up investigation.
- If any product affected by the unsatisfactory result is in distribution, the Food Recall and Emergency Response office must be informed.

14. Systematic review of reassessment of the HACCP system

The CFIA will conduct a systematic review 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 FSEP Specialist and the responsible Inspector/Veterinarian-in-Charge of the plant will review the control measures in consultation with the Area Program Specialist. They will complete CVS task 4401 and confirm that the information supplied by the Operator (e.g., HACCP Coordinator) is complete and reflects in-plant conditions.

The information collected will also be used when the CFIA updates sampling plans M-201 for the analysis of ground beef, and M-218 for the analysis of trim and raw beef components that may be used for the production of ground beef.

Appendix 1: Validation Procedure – Pathogen Reduction Step for Abattoirs

Sampling Protocol

The sampling should encompass a **four (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 *Enterobacteriaceae* (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 the values of EB counts before the intervention. The Input Variable 2 Range should correspond to after EB counts. The Hypothesized Mean Difference should be set to 0, and the Level of Signification (Alpha) to 0.05. Successful interventions should result in a P value inferior to 0.05.

Table 1: Number (n) of beef carcasses to select for the validation of microbiological reduction interventions based on indicator counts (STEP 2)

N	n for each Group		
	Group 1	Group 2	Group 3
100,000	15	133	237
50,000	15	133	236
25,000	15	133	235
10,000	15	132	231
5,000	15	130	226
1,000	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 beef carcasses to be sampled 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 (steam vacuum, organic acid sprays, etc.)

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

Table 2: Sample size to obtain a 95% confidence level that the product will be below 0.1% of *E. coli* O157:H7 during a one-month period (STEP 3)

Lot size (No. slaughtered per month)	Sample size (0.1% threshold)	Lot size (No. slaughtered per month)	Sample size (0.1% threshold)
10 - 69	All	1,900	1,507
70	All	2,000	1,552
80	All	2,100	1,595
90	All	2,200	1,636
100	All	2,300	1,674
110	All	2,400	1,711
120	All	2,500	1,745
130	All	2,600	1,778
140	All	2,700	1,809
150	All	2,800	1,839
160	All	2,900	1,867
170	All	3,000	1,894
180	All	3,200	1,945
190	All	3,500	2,012
200	All	3,800	2,072
300	All	4,200	2,141
400	All	4,600	2,201
500	499	5,100	2,265
600	596	5,700	2,329
700	690	5,800	2,339
800	781	6,700	2,415
900	868	7,900	2,492
1,000	950	9,700	2,576
1,100	1,028	12,400	2,660
1,200	1,101	17,200	2,748
1,300	1,170	28,200	2,841
1,400	1,235	77,300	2,937
1,500	1,296	100,000	2,950
1,600	1,354	125,000	2,959
1,700	1,408	Over 125 000	3,000
1,800	1,459	Over 125 000	3,000

Appendix 2. Testing Considerations for *E. coli* O157:H7
(As pertains to raw ground beef, raw ground beef patties, trim used in the production of ground beef, other components used to manufacture ground beef, and carcass surface meat used to produce this trim)

A. Sample pick-up

The collected sample must be representative of the lot being tested and must meet the screening methodology specifications.

B. Screening Methodology (optional - the lab may proceed directly to confirmation)

- Testing with screening methodology must be performed either:
    - in a lab accredited by the Standards Council of Canada (SCC) or another accreditation body that is a signatory to the International Laboratory Accreditation Cooperation (ILAC) Mutual Recognition Agreement (MRA) as conforming to the requirements of ISO/IEC 17025:2005 for specific tests; **OR**
    - in a non-accredited laboratory that the CFIA may audit, if deemed necessary to determine if the laboratory is proficient with the methods used and that there is an established quality system in place.

- A potential positive result will be considered definitive, i.e. to correspond to a confirmed positive test result for *E. coli* O157:H7 **unless** the sample proceeds to the confirmation method as described in Section C (Confirmation Methodology).

- An approved sampling methodology must be used and appropriately followed as directed by the CFIA Meat Programs Division.

- For ground beef/pattie, trim or trim components, five (5) sub-samples of 65 g each must be tested, representing a total of 325 g of meat product (for industry testing, this may be tested as one composite of 325 g, with one test result).

- The following additional requirements apply to establishments that are eligible to export to the United States:
    - The method used must include an enrichment in a selective broth medium.
    - The test method should be able to detect 0.23 colony forming units (cfu)/g in a 25 g sample of 75/25 (lean/fat) ground beef.

- Other federally registered establishments: any of the following screening methods or equivalent (see * below) are acceptable:

Assurance GDS	MFLP-16
Bax	MFLP-30
VIP	MFLP-87
Warnex	MFLP-12
DuPont LFD	MFLP-19
Assurance EHEC EIA	MFLP-81
Merck Singlepath	MFLP-82
Tecra Visual Immunoassay	MFLP-91
Polymyxin ELISA	MFLP-92
20 hour Reveal	MFLP-95

C. Confirmation Methodology Requirements

- The confirmation test must be done in an accredited laboratory (as described in section B, Screening Methodology).

- If the initial screening was done in a non-accredited laboratory: product must be shipped to an accredited laboratory according to the *Transportation of Dangerous Goods Regulations* – the sending laboratory must have the facilities and training required by the *Transportation of Dangerous Goods Regulations*.

- The potential positive enriched broth must be kept between 2 and 8°C and arrive in the accredited lab within 24 hours of obtaining the potential positive result. The accredited lab must start the confirmation procedure within 24 hours of the initial potential positive result.

- The confirmation test must be done from the same broth that was tested potentially positive by the screening test.

- The confirmation method used must be approved and include an Immuno-Magnetic Separation step.

- The following method or **equivalent** (see * below) is acceptable:

    - MFLP-80 (Health Canada Compendium of Analytical Methods)

- **When performing confirmation testing of samples submitted by a federally registered establishment listed as eligible to export to the US, MFLP-80 will be modified as described below:**

    - If the cultural method confirms the presence of typical, non-sorbitol fermenting *E. coli* O157, the following steps are required in addition to the requirements outlined in MFLP-80.

    - It is mandatory to perform either the serological test for antigen H7 or to confirm the presence or absence of the toxin with acceptable methodology (e.g. MFLP-83, MFLP-93, or equivalent [see * below]).

    - If the test performed (either H7 or the toxin test) is negative, it is required that both of these tests be performed.

    - If the test performed (either H7 or the toxin test) is positive, it will be concluded that the isolate is a confirmed verotoxin producing *E. coli* O157 positive and no further confirmatory testing is required.

    - If the tests performed (H7 and the toxin test) are both negative, proceed as follows.

NOTE: The following Polymerase Chain Reaction (PCR) tests for the presence of toxin genes and/or H7 genes will be done in CFIA laboratories or in private accredited laboratories using methodology approved by the CFIA.

If the tests for the toxin and H7 are negative, a test for the presence of the toxin genes (*stx*1 and/or *stx*2) with an acceptable PCR method will be performed. If the toxin genes are confirmed positive, then the sample is considered confirmed positive for *E. coli* O157:H7.

If the test for the toxin genes is negative, a PCR test for the presence of the H7 gene (*fli*C) will be performed. If the test for the H7 gene is confirmed positive, then the sample is considered confirmed positive for *E. coli* O157:H7.

If the H7 gene is not confirmed (assuming toxin and toxin gene, as well as H7 serology tests were negative), then the sample is considered to be negative for *E. coli* O157:H7.

Footnote

* - "**or equivalent**" - contact the CFIA, National Laboratory Operations, Executive Director, for the CFIA requirements to demonstrate equivalency of methodology not listed as acceptable in this document.