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Proposed Draft Guidelines for the Control of Shiga Toxin-Producing *Escherichia coli* (STEC) in Raw Beef, Fresh Leafy Vegetables, Raw Milk and Raw Milk Cheeses, and Sprouts

(Prepared by the Electronic Working Group chaired by Chile and co-chaired by United States of America, France, and New Zealand)

Codex Members and Observers wishing to submit comments on the discussion paper should do so as instructed in CL 2022/56/OCS-FH available on the Codex webpage/Circular Letters 2022: <https://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/>

BACKGROUND

1. The 50th Session of the Committee on Food Hygiene (CCFH50) agreed to start new work on Guidelines on the control of Shiga-toxin producing *Escherichia coli* (STEC) in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. An electronic working group (EWG) was established, co-chaired by Chile and the United States of America (USA), working via the Codex Forum and open to participation by all Codex Members and Observers.
2. CCFH51 considered the report of the EWG on the guidelines for the control of STEC and focused on giving guidance on the terminology to be used for each of the commodities covered by the Guidelines, as well as the request to JEMRA for scientific advice. CCFH51 agreed to return the draft to step 2/3 for redrafting and to establish an EWG, chaired by Chile and co-chaired by the USA, France, and New Zealand.
3. Since CCFH52 was postponed due to the COVID19 pandemic, the revised texts were distributed in April 2021 by CL 2021/35/OCS-FH for comments by Members and Observers, further revised, and then distributed for comments in December 2021 via CL 2021/63/OCS-FH. A Virtual Working Group (VWG) met immediately prior to CCFH52 to get input on specific issues related to the three annexes.
4. CCFH52 considered the report of the EWG and the VWG (CCFH52/CRD5) and agreed with the proposals made in CRD5 and that these should be incorporated in the further elaboration of the Guidelines. CCFH52 agreed to return the proposed draft document to Step 2/3 for redrafting and circulation for comments and to establish an EWG, chaired by Chile and co-chaired by the United States of America, France, and New Zealand, and working in English.

TERMS OF REFERENCE

5. The EWG was given the following terms of reference:
 - i. Update the General Section and the Annexes on Raw Beef, Fresh Leafy Vegetables, and Raw Milk and Raw Milk Cheeses, taking into consideration the written comments that were submitted through the OCS in response to the CL 2021/63/OCS-FH, and CRDs submitted at CCFH52, as well as the virtual working group (CRD5) and plenary session discussions at CCFH52.
 - ii. Draft an annex on Sprouts describing interventions relevant to control of STEC; and

- iii. Review the relevant JEMRA reports with respect to control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts and incorporate appropriate interventions and other changes into the annexes and general part as appropriate.

PARTICIPATION AND METHODOLOGY

6. An invitation was sent to all Codex Members and Observers to participate in the EWG; participants from 37 Codex member countries and 3 observer organizations registered for the EWG. The list of participants is attached as Appendix II. In addition to working through the Codex Forum, the EWG met virtually in June 2022 to resolve a number of issues.
7. The EWG redrafted the General Section, the Raw Beef Annex, Fresh Leafy Vegetables Annex and Raw Milk and Raw Milk Cheeses annex, based on written comments submitted to CCH52; comments received at the VWG (February 27 and June 8-9) and comments received through the Codex Forum.
8. The EWG developed an annex on Sprouts describing interventions relevant to control of STEC in these foods; provided the document on the forum for EWG member's input; and revised the documents based on EWG inputs.

SUMMARY OF DISCUSSION

9. The following changes were made in the documents after a round of consultation in the EWG and the Virtual Working Group meeting (June 2022).

10. General Section

- Made changes agreed to in the VWG meeting and changes suggested in comments received, including editorial changes.
- Noted in paragraph 1 of the introduction that STEC have occasionally been linked with neurological symptoms, including epileptic seizures and cognitive dysfunction.
- Deleted in paragraph 14 a footnote reference to "FAO/WHO 2009. Risk characterization of microbiological hazards in food. Microbiological risk assessment series 17." This reference was updated by "FAO/WHO 2021. Microbiological risk assessment: guidance for food (MRA 36)". However, the updated reference is not applicable to the statement about needing to validate under commercial conditions.
- Revised commodity definitions for consistency with those in the annexes. (The Sprouts definition is in square brackets pending agreement on definition by CCFH.)
- Added a statement in paragraph 32 that control measures proposed by food business operators (FBOs) based on risk assessment need to be validated.
- Revised the order of the paragraphs in section 11.2 (Laboratory Analysis Criteria for Detection of STEC) for better understanding.
- Revised paragraph 69 to explain what is meant by a "country's highest priority" and how this relates to corrective actions.

11. Raw Beef Annex

- A definition for tenderized raw beef was included in the annex. In the case of a definition for raw non-intact beef products, a footnote was inserted in paragraph 6 of the introduction instead of a definition since it is not mentioned in the document more than once.
- The flowchart step of bunging was arranged in a different order and the word "mechanical" was added before tenderization to avoid confusion with other means of tenderization.
- The word serotype was included every time *E. coli* O157:H7 was mentioned in the text.
- The term "High risk STEC" was changed to "STEC considered to be a country's highest priority." To provide clarity, the following text was inserted in parentheses to indicate which strains should be considered as such after the term is first mention, "e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country."

Question for CCFH53 with respect to the raw beef annex:

Do you think it relevant for the purpose of this document to add a “Post-Mortem inspection step“ to this flow diagram between Splitting and Carcass Washing?

12. Fresh Leafy Vegetables Annex

- Made changes agreed to in the VWG meeting and changes suggested in comments received, including editorial changes.
- Deleted references.
- In paragraph 10 added square brackets around the following statement pending the JEMRA report: “[Once product is contaminated with STEC it is not possible to eliminate it and there are limited control measures that can be implemented to reduce it.]” (Note that paragraph 9 similarly says “The assessment of environmental conditions is particularly important because subsequent interventions would not be sufficient to fully remove STEC contamination that occurs during primary production...”)
- In paragraph 15, revised the first sentence and added square brackets pending the JEMRA report: “[Growers should periodically test the water they use for appropriate indicator microorganisms and, where necessary, STEC,] according to the risk associated with the production.”
- Revised the flow diagram to use dotted lines instead of color around two boxes and added asterisks with a footnote that “Boxes with broken lines indicate steps that may not be included, depending in part on the commodity.”

Questions for CCFH53 with respect to the Fresh Leafy Vegetables Annex:

- In paragraph 2, we say that “There is no processing treatment applied that would eliminate or inactivate STEC, although contamination can be reduced by washing in water containing antimicrobials.” One comment asked about ozone treatments. Should we say that “...contamination can be reduced by treatments such as washing in water containing antimicrobials?” Is there something we should add about ozone based on information from JEMRA?
- The definition of Fresh Leafy Vegetables refers to those intended for consumption without cooking. However, there are processes other than cooking that can adequately reduce microbial pathogens. JEMRA has defined “fresh fruits and vegetables” as “Fruits and vegetables that are not processed in a manner that changes their physical properties. Cooked, canned, juiced, frozen, candied, dried, pickled, fermented, or otherwise preserved foods derived from fruits and vegetables were excluded from this definition and this report.” In this annex we only refer to “cooking,” but in the *Code of Hygienic Practice for Fresh Fruits and Vegetables*, Annex III “fresh leafy vegetables,” the scope refers to those “intended to be consumed without further microbiocidal steps” (terminology also used in the definition of ready-to-eat fresh fruits and vegetables). Do we need to consider other processes and say, “for consumption without any further microbiocidal steps” instead of “for consumption without cooking”?

13. Raw Milk and Raw Milk Cheeses Annex

- Made changes agreed to in the VWG meeting and changes suggested in comments received, including editorial changes.
- Changed the format of the text to remove the “scientific knowledge” parts
- Removed references
- Changed in the two diagrams at the end of the document:
 - a. For the flow diagram in figure 1 (entitled “Process Flow Diagram for Raw Milk Production, Distribution and Sale”):
 - i. Added “Raw” before milk in the box “Milk collection and transport” (3rd box from the top)
 - ii. Added “Raw” before milk in the box “Milk” (box on the left of the figure).
 - b. For the flow diagram in figure 2 (entitled “Making Cheese from Raw Milk”):

- i. Added "Raw" before milk in the box "Milk" (box on the left of the figure and 3rd box from the top).
- ii. Added a dotted arrow from "receive raw milk" to "addition of ingredients" (Some cheeses are made directly without cold storage)

- Revised what is meant by a "country's highest priority" and how this relates to corrective actions to be consistent with paragraph 69 in the general section.

14. Sprouts

- Several suggestions from members and observers were incorporated to the text after a round of consultation to the EWG.
- The scope of the annex was set as specific guidance for the control of STEC related to sprouts that are intended for human consumption without cooking. Home-sprouting, and shoots, cress, and microgreens where the seed is not kept in the final product are outside the scope of this document. However, questions have been posted by members at the Codex Forum whether microgreens should be part of this annex or the leafy vegetables annex. It was decided that this should be discussed at CCFH53.
- Regarding the physical and chemical treatments mentioned in the annex, one member indicated that we must include the quantity and concentration for these treatments in the annex. This is not a common practice, but the co-chairs believe it would be useful, and decided that this should be discussed at CCFH53.

Question for CCFH 53 with respect to the Sprouts Annex:

- In paragraph 48 there are several chemical treatments mentioned. Since scientific references will be deleted in a later step of the document, should we include the concentrations that were shown in the referenced studies to achieve the log reduction (after JEMRA validation)?
- In paragraph 49 there are several physical treatments mentioned. Do you think it would be useful include examples (e.g., time and temperature) for each one of the treatments recommended (after JEMRA validation)?
- Microgreens share characteristics with sprouts. They have the same initial process and steps, originate from similar seeds, and seed contamination will spread similarly. However, STEC outbreaks have not been associated with them to date. Should we include microgreens under the scope of this annex?

CONCLUSIONS

15. The EWG completed the tasks identified in its Terms of Reference; specifically, the EWG:
- a. Updated the General Section and the Annexes on Raw Beef, Fresh Leafy Vegetables, and Raw Milk and Raw Milk Cheeses, taking into consideration the written comments that were submitted through the OCS in response to the CL 2021/63/OCS-FH, and CRDs submitted at CCFH52, as well as the virtual working group (CRD5) and plenary session discussions at CCFH52.
 - b. Drafted an annex on Sprouts describing interventions relevant to control of STEC.
 - c. Reviewed the relevant JEMRA summary reports with respect to control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts and incorporated appropriate interventions and other changes into the annexes and general part as appropriate. (Note: the full JEMRA report was not available at the time the EWG completed its work.)

RECOMMENDATIONS

16. The EWG recommends that CCFH53:
- a. consider the proposed draft Guidelines as presented in Appendix I, including the General Section, Annex 1 (Raw Beef), Annex 2 (Fresh Leafy Vegetables), Annex 3 (Raw Milk and Raw Milk Cheeses) and Annex 4 (Sprouts),
 - b. respond to the specific questions asked above, and provide suggestions and comments for revision, and
 - c. recommend whether the document can be advanced in the Codex Step process.

APPENDIX I

GUIDELINES FOR THE CONTROL OF SHIGA TOXIN-PRODUCING *E. COLI* (STEC) IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW MILK CHEESES, AND SPROUTS**1. INTRODUCTION**

1. Shiga toxin-producing *Escherichia coli* (STEC) are recognized as foodborne pathogens, causing human illnesses with a wide range of mild to severe gastrointestinal presentations from asymptomatic to diarrhoea to bloody diarrhoea, occasionally leading to severe hemolytic uremic syndrome with kidney failure and death. STEC have occasionally been linked with neurological symptoms, including epileptic seizures and cognitive dysfunction. Strains of *E. coli* that are pathogenic to humans have been classified into several groups, and STEC are defined by the potential to produce one or more Shiga toxins. STEC strains are a diverse group which can cause disease in humans. STEC strains that can cause hemorrhagic colitis may be referred to as enterohemorrhagic *E. coli* (EHEC). The most well-studied and documented STEC serotype is *E. coli* O157:H7. The burden of the disease and the cost of control measures are significant; STEC outbreaks have been associated with diverse food commodities, and thus STEC have the potential to have a serious impact on public health.

2. Clinical symptoms of the disease in humans arise as a consequence of consuming food contaminated with *E. coli* that produces Shiga toxin type 1 (Stx1) (encoded by the gene *stx1*) and/or Shiga toxin type 2 (Stx2, encoded by the gene *stx2*). Historically, the term verotoxin has also been used for the Shiga toxins of *E. coli* and the term verotoxigenic *E. coli* (VTEC) used synonymously with STEC. In this document, the term “Shiga toxin” (Stx) is used to indicate the protein toxin, “*stx*” to indicate the toxin gene, and “STEC” to indicate the *E. coli* strains demonstrated to carry *stx* and produce Stx. STEC are pathogenic to humans after ingestion and attachment to the intestinal epithelial cells where production of Stx occurs. Attachment to intestinal epithelial cells is the result of other proteins, including the principal adherence protein intimin, encoded by *eae*. The aggregative adherence fimbrial adhesins commonly associated with enteroaggregative *E. coli*, regulated by the *aggR* gene, when found in isolated strains with *stx*, have also been linked to severe illness and have been used as predictors of pathogenicity. (Table 1 shows combinations of virulence genes and their association with disease severity that can be used for risk management purposes.) There may be additional genes involved in pathogenicity that have not been identified yet. Some of these virulence genes are located on mobile genetic elements (e.g., plasmids, bacteriophages, pathogenicity islands) and can be horizontally transmitted to related microorganisms or be lost. Symptoms and their severity of the disease are determined by the variability in the virulence genes, among other factors such as gene expression, dose, host susceptibility, and age. Because STEC are primarily a genotype-based hazard, this has implications for hazard identification and characterization, which will be discussed in these Guidelines.

3. Historically, illnesses caused by STEC have been linked to the consumption of raw or undercooked ground/minced or tenderized beef; however fresh leafy vegetables, sprouts, and dairy products (raw milk and raw milk cheeses) have been increasingly recognized as commodities that pose a risk of illness from STEC. Sources of STEC in these foods can vary, as does the ability of the organism to survive and multiply within them. The association of specific food categories with STEC illness reflects the historical and current practices of food production, distribution and consumption. Changes in food production, distribution and consumption can cause changes in STEC exposure. Consequently, microbial risk management should be informed by an awareness of current local sources of STEC exposure. This guidance document will identify commodity-specific intervention practices based on known source attribution in these different foods, and practices for monitoring STEC in food products, including the utility of indicator microorganisms.

4. It is generally accepted that animals, in particular ruminants, are the primary reservoir/source of STEC. STEC-positive ruminants are typically asymptomatic. Contamination with intestinal content or faeces is the most likely initial source of STEC in most foods. For example, STEC outbreaks have been associated with raw beef contaminated with STEC during the slaughtering process, field-grown fresh leafy vegetables have been linked to STEC-contaminated irrigation water, and STEC illnesses from sprouts have resulted from contamination during seed production enhanced during sprouting. Raw milk is most commonly contaminated as a result of soiled udders and teats, as well as poor hygiene during milking.

5. The large degree of variation exhibited by STEC in their biological properties, host preferences, and environmental survival presents a challenge for managing the presence of STEC in animal and plant production. In practice, this means that there is no “one size fits all” solution, and different production systems may require different approaches to control the various serotypes of STEC (such as approaches based on pathogenicity and ability to cause severe illness). In most instances, control measures will reduce STEC but not eliminate them.

6. These Guidelines build on general food hygiene provisions already established in the Codex system and propose potential control measures specific for STEC strains in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts.
7. Examples of control measures in each commodity-specific annex have been subjected to a scientific evaluation by the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) in development of the Guidelines. Such examples are illustrative only and their use and approval may vary among member countries.
8. The format of this document:
 - Provides an opening general section with STEC guidance applicable to all commodities.
 - Demonstrates the range of the approaches of control measures for STEC.
 - Facilitates development of hazard analysis and critical control points (HACCP) plans at individual establishments and at national levels.
 - Assists in assessing the equivalence¹ of control measures for raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts applied in different countries.
9. The Guidelines provide flexibility for use at the national (and individual processing) level.

2. OBJECTIVES

10. These Guidelines provide information to governments and food business operators (FBOs) on the control of STEC that aims to reduce foodborne disease from raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. They provide a [science-based and practical] tool for the effective control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts according to national risk management decisions. The control measures that are selected can vary among countries and production systems.
11. These Guidelines do not set quantitative limits as described in the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997) for STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. Rather, the Guidelines describe control measures that countries can establish as appropriate to their national situation as described in the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

3. SCOPE AND USE OF THE GUIDELINES

3.1. Scope

12. These Guidelines are applicable to STEC that may contaminate raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts and cause foodborne disease. The primary focus is to provide information on scientifically validated practices that may be used to prevent, reduce, or eliminate STEC contamination of raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts.

3.2. Use

13. The Guidelines provide specific control measures for STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts according to a primary production-to-consumption food chain approach, with potential control measures being identified at applicable steps in the process flow. The Guidelines are supplementary to and should be used in conjunction with the *General Principles of Food Hygiene* (CXG 1-1969), the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004), the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008), and *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007). These general and overarching provisions are mentioned as appropriate, and their content is not duplicated in these Guidelines.
14. The Guidelines present a number of control measures. These control measures will likely vary at the national level and therefore these Guidelines only provide examples of them. Examples of control measures are limited to those that have been scientifically demonstrated as effective in a commercial setting. Countries should note that these control measures are indicative only. The quantifiable outcomes reported for control measures are specific to the conditions of particular studies and the control measures would need to be validated under local commercial

¹ *Guidelines on the Judgement of Equivalence of sanitary Measures Associated with Food Inspection and Certification Systems* (CXG 53-2003)

conditions to provide an estimate of hazard reduction. Government and FBOs can use choices on hazard-based control measures to inform decisions on critical control points (CCPs) when applying HACCP principles to a particular food process.

15. Several control measures as presented in these Guidelines are based on the use of physical, chemical and biological decontamination processes to reduce the prevalence and/or concentration of STEC-positive commodities, for example decontamination of beef carcasses from slaughtered cattle (i.e., beef from animals of the species of *Bos indicus*, *Bos taurus*, and *Bubalus bubalis*). The use of these control measures is subject to approval by the competent authority, where appropriate, and varies based upon the type of product being produced. Also, these Guidelines do not preclude the choice of any other control measure that is not included in the examples provided herein, and that may have been scientifically validated as being effective in a commercial setting.

16. The provision of flexibility in the application of the Guidelines is an important attribute. They are primarily intended for use by government risk managers and FBOs in the design and implementation of food safety control systems.

17. The Guidelines should be useful when assessing whether different food safety measures for raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts in different countries are appropriate.

4. DEFINITIONS

18. For the purposes of these Guidelines, the following terms are defined as below:

19. Fresh leafy vegetables - Vegetables of a leafy nature where the leaf is intended for consumption without cooking, including, but not limited to, all varieties of lettuce, spinach, cabbage, chicory, endive, kale, radicchio, and fresh herbs such as coriander, cilantro, basil, curry leaf, colocasia leaves and parsley, among other local products for foliar consumption.

20. Indicator microorganisms - microorganisms used as an indicator of quality, process efficacy, or hygienic status of food, water, or the environment, commonly used to suggest conditions that would allow the potential presence or proliferation of pathogens, a failure in process hygiene or in food processing. Examples of indicator microorganisms include mesophilic aerobic bacteria, coliforms or faecal coliforms, *E. coli* and Enterobacteriaceae.

21. Monitor: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control. 2

22. Raw beef: Skeletal muscle meat from slaughtered cattle, including primal cuts³, sub-primal cuts, and trimmings.

23. Raw milk: Milk (as defined in the *General Standard for the Use of Dairy Terms* (CODEX STAN 206-1999)) which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect.^{4, 5, 6}

24. Raw Milk Cheeses: Cheeses made from raw milk.

25. Shiga Toxin-Producing *E. coli* (STEC): A diverse group of pathogenic bacterial strains of *Escherichia coli* that are demonstrated to carry Shiga toxin genes (*stx*) and produce Shiga toxin protein (Stx).

26. [Sprouts: Sprouted seeds or beans harvested when the cotyledons (or seed leaves) are still un- or underdeveloped and true leaves have not begun to emerge. They can be grown in water, soil or substrate and can be harvested with or without the root (cut sprouts)⁷]

² *General Principles of Food Hygiene* (CXG 1-1969)

³ A primal cut is a piece of meat on the bone initially separated from the carcass of an animal during butchering. Primal cuts are then divided into sub-primal cuts. These are basic sections from which steaks and other subdivisions are made

⁴ *The Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004)

⁵ Heat treatment beyond 40°C results in changes such that the structure of the resultant product is no longer the same as that of raw milk. In addition, a point temperature of 40°C, and up to pasteurization temperatures, is generally considered to be insufficient to kill STEC in raw milk.

⁶ Milk that has been subject to processing techniques such as microfiltration and/or bacto-fugation is no longer considered raw milk.

⁷ FAO/WHO. 2022. Microbiological Risk Assessment Series 43: *Prevention and control of microbiological hazards in fresh fruits and vegetables – sprouts*.

27. Validation of control measures: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.⁸

28. Verification: The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.⁹

5. PRINCIPLES APPLYING TO CONTROL OF STEC IN RAW BEEF, FRESH LEAFY VEGETABLES, RAW MILK AND RAW MILK CHEESES, AND SPROUTS

29. Overarching principles for good hygienic practice for meat production are presented in the *Code of Hygienic Practice for Meat* (CXC 58-2005), Section 4: General Principles of Meat Hygiene. For fresh leafy vegetables and sprouts, overarching principles for good hygienic practice are presented in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), Annex I on Ready-To-Eat Fresh Pre-Cut Fruits and Vegetables and Annex III on Fresh Leafy Vegetables. Additionally, see the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) for dairy products. Two overarching food safety principles that have particularly been taken into account in these Guidelines are:

a) The principles of food safety risk analysis¹⁰ should be incorporated wherever possible and appropriate in the control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts from primary production-to-consumption.

b) Wherever possible and practical, competent authorities should formulate risk management metrics¹¹ so as to objectively express the level of control of STEC in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts that is required to meet public health goals (including focusing on subtypes of particular concern where appropriate).

6. PRIMARY PRODUCTION-TO-CONSUMPTION APPROACH TO CONTROL MEASURES

30. These guidelines incorporate a “primary production-to-consumption” flow approach that identifies the main steps in the food chain where control measures for STEC can potentially be applied in the production of each commodity. The systematic approach to the identification and evaluation of potential control measures allows consideration of the use of controls in the food chain and allows different combinations of control measures to be developed and implemented. This is particularly important where differences occur in primary production and processing systems among countries. Risk managers need the flexibility to choose risk management options that are appropriate to their national context.

31. Good Hygiene Practices (GHPs) provide the foundation for most food safety control systems. Where possible and practicable, food safety control measures for STEC should incorporate hazard analysis activities and appropriate control measures. Identification and implementation of risk-based control measures based on risk assessment can be elaborated by application of a risk management framework process as advocated in the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

32. While these Guidelines provide generic guidance on development of control measures for STEC, development of risk-based control measures for application at a single step or at multiple steps in the food chain are primarily the domain of competent authorities at the national level. FBOs can select the risk-based measures to facilitate the effective application of process control systems and comply with the requirements of the competent authority. When no microbiological criteria or food safety objectives have been established by competent authorities, FBOs are also able to propose control measures based on risk assessment. These control measures need to be validated.

33. Specific control measures for STEC are described in each commodity-specific annex, where appropriate: Annex I – raw beef; Annex II - fresh leafy vegetables; Annex III – raw milk and raw milk cheeses; Annex IV - sprouts.

6.1 Development of risk-based control measures

34. Competent authorities operating at the national level should, working with the relevant food sector, develop risk-based control measures for STEC where possible and practical.

⁸ *General Principles of Food Hygiene* (CXG 1-1969)

⁹ *General Principles of Food Hygiene* (CXG 1-1969)

¹⁰ *Working Principles for Risk Analysis for Food Safety for Application by Governments* (CXG 62-2007)

¹¹ *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007)

35. Risk modelling tools can be developed¹² to assess the impact of control measures on the prevention, reduction, or elimination of the hazard. The capability and limitations, including the need for quantitative data, of the tools should be clearly specified and understood by the risk manager.

36. Competent authorities formulating risk management metrics¹³ as regulatory control measures should apply a methodology that is scientifically robust and transparent.

7. PRIMARY PRODUCTION CONTROL MEASURES

37. Controls in the primary production phase of the process flow are focused on decreasing the number of animals that are carrying and/or shedding STEC, as well as preventing or reducing plants being contaminated with STEC on the farm. In addition, Good Agricultural Practices (GAPs) and animal husbandry practices related to water, worker hygiene, appropriate use of fertilizers and biosolids, appropriate handling during transport, temperature control, and cleanliness of contact surfaces can reduce the incidence of STEC at primary production.

8. PROCESSING CONTROL MEASURES

38. Appropriate controls to prevent and/or reduce the contamination and cross contamination by STEC of commodities during processing are important. Control measures during post-processing handling and storage are also important to prevent growth of and cross contamination with STEC.

9. FOOD DISTRIBUTION CONTROL MEASURES

39. Control measures during distribution to ensure product is stored at an appropriate temperature to prevent growth of STEC beyond a detectable level and to minimize cross contamination by STEC are important.

10. IMPLEMENTATION OF CONTROL MEASURES

40. Implementation¹⁴ involves giving effect to the selected control measure(s), development of an implementation plan, communication of the decision on control measure(s), ensuring a regulatory framework and infrastructure for implementation exists, and a monitoring and evaluation process to assess whether the control measure(s) have been properly implemented.

10.1 Prior to Validation

41. Prior to validation of the control measures for STEC, the following tasks should be completed:

- Identification of the specific measure or measures to be validated. This would include analysis of any measures agreed to by the competent authority and whether any measure has already been validated in a way that is applicable and appropriate to specific commercial use, such that further validation is not necessary.
- Identification of any existing food safety outcome or target established by the competent authority or FBOs. In order to comply with the target set by the competent authority, FBOs may set stricter targets than those set by the competent authority.

10.2 Validation

42. Validation of control measures may be carried out by FBOs and/or the competent authority. [Validation of control measures should be performed based on the capacity of the control measures to decrease the risk for public health.]

43. Where validation is undertaken for a measure to control STEC, evidence will need to be obtained to show that the measure is capable of controlling STEC to a specified target or outcome. This may be achieved by use of a single measure or a combination of control measures. The *Guidelines for the Validation of Food Safety Control Measures* (CXG 69-2008) (Section VI) provides detailed advice on the validation process.

10.3 Implementation of validated control measures

44. Refer to Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004).

¹² *Principles and Guidelines for the Conduct of Microbiological Risk Assessment* (CXG 30-1999)

¹³ *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007)

¹⁴ See Section 7 of the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

10.3.1 FBO responsibility

45. FBOs have the primary responsibility for implementing, documenting, validating, verifying and supervising process control systems to ensure the safety and suitability of raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts. These should incorporate measures for control of STEC as appropriate to national government requirements and the FBO's specific circumstances, and where applicable the measures should be applied in accordance with manufacturer's instructions.

46. The documented control measures should describe the activities applied, including any sampling procedures, specified targets (e.g., performance objectives or performance criteria) set for STEC, FBO verification activities, and corrective actions.

10.3.2 Regulatory systems

47. The competent authority, working with the relevant food sector, may provide guidelines and other implementation tools to FBOs, as appropriate, for the development of the process control systems.

48. The competent authority may assess the documented process control systems to ensure they are science based and establish verification frequencies. Microbiological testing programmes, or molecular testing programmes, should be established to verify the effectiveness of control measures for STEC.

10.4 Verification of control measures

49. Refer to Section 9.2 of the *Code of Hygienic Practice for Meat* (CXC 58-2005), the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004), and Section IV of the *Guidelines for the Validation of Food Safety Control Measures* (CXG 69 -2008).

10.4.1 Food Business Operators

50. FBOs may use testing information on indicator microorganisms for verification of STEC control measures due to the high cost of testing for detection of STEC and its low prevalence in food. FBO verification activities should verify that all control measures for STEC have been implemented as intended. Verification should include observation of monitoring activities (such as having an employee with overall responsibility for monitoring activities observe the person conducting a monitoring activity at a specified frequency), reviewing monitoring, corrective action and verification records, and sampling and testing for indicator microorganisms and STEC where appropriate.

51. Due to typically low numbers and low prevalence of STEC in food, quantitative monitoring of STEC is impractical and the utility of presence/absence testing in monitoring process performance is also limited (FAO/WHO 2018). Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring sanitary and hygiene indicator microorganisms. These indicator microorganisms do not indicate pathogen presence or absence; instead, they provide a quantitative measure of the control of general microbial contamination in the product and processing or growing environment. The hygiene indicator microorganisms used should be those that are the most informative for the specific processing or growing environment. An increase in the number of the indicator microorganism above established control values indicates a loss of control and the need for corrective action. Additionally, with the increase in the frequency of verification, there is also an increase in the speed of detecting a loss of control of manufacturing hygiene. Verification at multiple points in the processing chain can assist in rapid identification of the specific process step where corrective action should be taken. Monitoring of hygiene indicator microorganisms can be supplemented by periodic testing for STEC where appropriate and as needed to make risk-based decisions. STEC testing can contribute to reducing contamination rates, improving food safety, and promoting continuous process improvement, if testing results are linked to requirements for corrective action.

52. Verification frequency could vary according to the operational aspects of process control, the historical performance of the establishment, and the results of verification activity itself.

53. Record keeping is important to facilitate verification and for traceability purposes.

10.4.2 Regulatory systems

54. The competent authority should verify that all regulatory control measures implemented by FBOs comply with regulatory requirements, as appropriate, for control of STEC.

11. MONITORING AND REVIEW

55. Monitoring and review of food safety control systems is an essential component of the application of a risk management framework¹⁵. It contributes to verification of process control and demonstrating progress towards achievement of public health goals. Effective monitoring includes verifying the effectiveness of STEC control processes throughout the food chain.

56. Information on the level of control of STEC at appropriate points in the food chain can be used for several purposes, e.g., to validate and/or verify outcomes of food control measures, to monitor compliance with regulatory goals for STEC control, and to help prioritize regulatory efforts to reduce foodborne illness. Systematic review of monitoring information allows the competent authority and relevant stakeholders to make decisions in terms of the overall effectiveness of the food safety control systems and make improvements where necessary.

11.1 Monitoring

57. Monitoring via sampling and testing should be carried out at appropriate steps throughout the food chain using a validated diagnostic test and randomized or targeted sampling as appropriate.

58. For instance, the monitoring programmes for STEC and/or indicator microorganisms, when appropriate, in raw beef, fresh leafy vegetables, raw milk and raw milk cheeses, and sprouts may include testing at the farm (e.g., for fresh leafy vegetables), in the slaughter and processing establishments, and the retail distribution chains where appropriate and according to the monitoring objective.

59. Competent authority regulatory monitoring programmes should be designed in consultation with relevant stakeholders, where appropriate, and should consider the sampling plan, including the number, location, collection and testing of samples and resource constraints. Given the importance of monitoring data for risk management activities, sampling and testing components of regulatory monitoring programmes should be standardized on a national basis and be subject to quality assurance.

60. The type of samples and data collected in monitoring systems should be appropriate for the outcomes sought. Enumeration and further characterization of microorganisms generally provide more information for risk assessment and risk management purposes than presence/ absence testing. Where the regulatory monitoring program is to be carried out by FBOs, there should be flexibility with respect to the procedures used, as long as the FBO procedures provide equivalent performance to regulatory procedures.

61. Monitoring information should be made available to relevant stakeholders in a timely manner (e.g., where appropriate, to producers, FBOs, competent authorities, the public health sector, and consumers).

62. Monitoring information collected from throughout the food chain should be used to affirm achievement of risk management goals. Wherever possible, such information should be combined with human health surveillance data and foodborne illness source attribution data to validate risk-based control measures and verify progress towards risk-reduction goals.

11.2 Laboratory Analysis Criteria for Detection of STEC

63. The choice of analytical method should reflect both the type of sample to be tested and the purpose for which the data collected will be used. The purpose of analysis for bacterial foodborne pathogens, including STEC, can be divided into the following categories:

- product batch or lot acceptance;
- process performance control to meet domestic food regulation;
- to verify controls to meet market access requirements (e.g., to meet microbiological criteria of another country); and
- public health investigations.

64. The number of foods identified as a vehicle for STEC transmission has increased over time. Baseline studies and targeted surveys are conducted to provide prevalence data and identify risk factors along the food chain. These data, together with public health surveillance data, are used in risk assessments and risk profiles of STEC/ food combinations to prioritize foods and STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country). Analytical methods that are fit for purpose, that will provide answers to risk management

¹⁵ See Section 8 of the *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)* (CXG 63-2007).

questions, and that are within the resources of governments and FBOs should be chosen.¹⁶ In the event that a laboratory does not have the resources and technology to characterize the isolate, it could be sent to a reference centre/ laboratory.

65. The risk of severe illness due to STEC infection can be predicted according to virulence factors (encoded by genes) present in an STEC strain, and testing for such factors should be used as complementary data to assess and predict the virulence potential of STEC strains recovered from food samples. Based on current scientific knowledge, all STEC strains are pathogenic for humans and capable of causing illness. However, STEC strains with *stx2a* and adherence genes, *eae* or *aggR*, have the greatest association with severe illness such as bloody diarrhoea (BD), haemolytic uremic syndrome (HUS) and hospitalizations. Thus, to appropriately manage the risk of STEC in commodities discussed in this guidance document, tests that detect virulence factors such as these should be used. The risk of severe illness may also depend on virulence gene combinations and gene expression, the dose ingested, and the susceptibility of the human host, so a risk management framework should also be applied when laboratory methodologies for STEC detection are selected by countries.

66. The severity of STEC illness and the potential to cause diarrhoea, bloody diarrhoea and haemolytic uremic syndrome, hence the degree of public health relevance, can be defined by the combination of virulence genes within an isolated strain of STEC. These combinations can be ranked from the most severe (1) to least severe (5), and are recommended by JEMRA¹⁷ as criteria (Table 1) for developing risk management goals that prioritize:

- the STECs of greatest public health relevance,
- the design of monitoring and surveillance programmes by competent authorities, and
- resourcing public health investigations and recalls in response to a positive test.

67. The JEMRA report notes that the association of Stx subtypes other than Stx2 with HUS is less conclusive and varies depending on other factors, for example host susceptibility, pathogen load, and antibiotic treatment.

Table 1. STEC virulence genes in isolated strains and the potential to cause diarrhoea (D), bloody diarrhoea (BD) and haemolytic uremic syndrome (HUS) (where 1 is the highest risk level). *

LEVEL	TRAIT (GENE)	POTENTIAL FOR
1	<i>stx2a</i> + <i>eae</i> or <i>aggR</i>	D/BD/HUS
2	<i>stx2d</i>	D/BD/HUS**
3	<i>stx2c</i> + <i>eae</i>	D/BD [^]
4	<i>stx1a</i> + <i>eae</i>	D/BD [^]
5	Other <i>stx</i> subtypes	D [^]

* depending on host susceptibility or other factors; e.g. antibiotic treatment

**association with HUS dependent on *stx2d* variant and strain background

[^] some subtypes have been reported to cause BD, and on rare occasions HUS

68. The determination of virulence and other salient marker genes for testing purposes may be achieved by using, for example, polymerase chain reaction (PCR) methods or whole genome sequencing (WGS) analysis on isolated strains. Special consideration should be given to the efficacy of sample collection techniques to maximize portions of product most likely to be contaminated. The choice of enrichment culture techniques used to recover STEC from foods is also important, as STEC strains are physiologically diverse, with variable growth characteristics. Selective conditions which are permissive to specific sub-populations of STEC, such as *E. coli* O157:H7, can be used, but this poses the risk of inhibiting the multiplication of other STEC strains, preventing their detection.

69. In addition, bacteria other than STEC may contain the same virulence genes and the detection of these genes alone may not fully reflect health risk due to differential or lack of gene expression. It is also very important to characterize STEC isolates. The isolation of STEC by immunomagnetic separation (IMS) or by traditional culture-based methods is essential to confirm presumptive PCR positive samples.

70. Consideration of virulence genes plays a role in the management of STEC in food commodities, including the actions to be taken when STEC is detected in the food. As shown in Table 1, different combinations of virulence genes create differences in risk for severe illness, but factors other than the virulence genes also play a role. The

¹⁶ FAO/WHO. 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological Risk Assessment Series No. 31. Rome. Available at <http://www.fao.org/3/ca0032en/ca0032en.pdf>.

¹⁷ FAO/WHO. 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological Risk Assessment Series No. 31. Rome. Available at <http://www.fao.org/3/ca0032en/ca0032en.pdf>.

priority of STEC strains carrying specific virulence genes varies from country to country, and, thus, the corrective actions needed on finding STEC in a food will also vary by country. In general, more stringent corrective actions would be applied for STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country) than for those that are a lower priority.

11.3 Review

71. Periodic review of monitoring data for STEC at relevant process steps should be used to inform the effectiveness of risk management decisions and actions, as well as future decisions on the selection of specific control measures for STEC and provide a basis for their validation and verification.

72. Information gained from monitoring for STEC in the food chain should be integrated with human foodborne disease surveillance, food source attribution data, and withdrawal and recall data, where available, to evaluate and review the effectiveness of STEC control measures from primary production to consumption.

73. Where monitoring of hazards or risks indicates that regulatory performance goals are not being met, risk management strategies and/or control measures should be reviewed.

11.4 Public health goals

74. Competent authorities should consider the results of monitoring and review when reevaluating and updating public health goals for control of STEC in foods, and when evaluating progress. Monitoring of food chain information in combination with data on food source attribution and human health surveillance is an important component. The surveillance and application of controls for the proper functioning of the STEC control systems need to ensure that the food chain is sufficiently safe for human health.

RAW BEEF

1. INTRODUCTION

1. Foodborne outbreaks of Shiga toxin-producing *Escherichia coli* (STEC) have been linked to a wide variety of foods, including meat products. Beef is one of the most significant sources of foodborne STEC outbreaks, with raw or undercooked non-intact raw beef products (e.g., ground/minced, or tenderized beef) recognised as posing an elevated risk to consumers.
2. Grinding/mincing and processes such as marinating, in combination with knife scoring, proteolytic enzymes, or vacuum brine injection, and mechanical tenderisation in which blades or needles penetrate the muscle surface, create a potential for increased food safety risks due to the transfer of pathogens from the surface to the interior, resulting in internalization of STEC into previously intact raw beef.
3. STEC can be part of the normal intestinal microbiota of cattle, with the reported prevalence in cattle faeces varying greatly, depending on factors such as animal age, herd type, season, geographic location, and production type. STEC shedding by individual cattle is transient and episodic. In addition, STEC can be found within the farm environment, and it is therefore likely that cattle arriving for slaughter have STEC on their hides. Individual feedlot cattle studies have reported the prevalence of STEC O157 on cattle hides presenting for slaughter as high as 94.5%, and as high as 74.5% for other STEC.
4. The sporadic nature of STEC and common movement and comingling of cattle through means such as feedlots, lairage, and livestock markets allows STEC to spread between animals. The transient nature of STEC in cattle and the impracticality of testing all cattle for STEC prior to slaughter demonstrate the need for slaughter operations to treat all incoming cattle as if they could have STEC on the hide or could be shedding STEC in their faeces.
5. STEC carried by cattle could be spread to carcasses during slaughter. Prior to slaughter, the muscle tissue of healthy cattle is free of STEC. STEC can be transferred to carcass surfaces from the contents of the gastrointestinal tract or hide during the operations of dehiding, head removal, bunning and evisceration. Generally, contamination is confined to the carcass surface and is not found in deep muscle tissues of intact raw beef.
6. STEC contamination has historically been detected in non-intact raw beef products¹⁸. The purpose of this guidance is to provide information on measures that can reduce contamination of raw beef with STEC and guidance on when raw beef contaminated with STEC should be considered fit for human consumption to minimize the potential for disputes and facilitate global trade.

2. SCOPE

7. This guidance applies to control of STEC in raw beef, including non-intact products such as raw ground/minced or tenderized beef.
8. This guidance does not apply to raw beef meat preparations (raw beef meat which has had foodstuffs, seasonings or additives added to it).

3. DEFINITIONS

9. For the purpose of this guideline the following definitions apply:

Raw Beef: Skeletal muscle meat from slaughtered cattle, including primal cuts¹⁹, sub-primal cuts, and trimmings.

Minced beef: Boneless beef which has been comminuted, i.e., reduced into fragments.²⁰

¹⁸ Non-intact raw beef products include comminute beef products such as those that are ground or minced, as well as those that have been mechanically tenderized. They can also include raw beef that has been injected/enhanced with solutions or reconstructed into formed entrees (e.g., beef that has been scored to incorporate a marinade, beef that has a solution of proteolytic enzymes applied to or injected into the cut of meat, or a formed and shaped product such as beef gyros), but these non-intact beef products are out of scope for this document.

¹⁹ A primal cut is a piece of meat on the bone initially separated from the carcass of an animal during butchering. Primal cuts are then divided into sub-primal cuts. These are basic sections from which steaks and other subdivisions are made.

²⁰ Adapted from the *Code of Hygienic Practice for Meat* (CXC 58-2005).

Tenderized raw beef²¹: Cuts of beef that have gone through a technological process for the rupture of muscle fibers by mechanical action with small blades or needles which penetrate the muscle surface thereby resulting in tenderizing.

4. PRIMARY PRODUCTION-TO-CONSUMPTION APPROACH TO CONTROL MEASURES

10. These Guidelines incorporate a “primary production-to-consumption” flow diagram that identifies the main steps in the food chain and identifies where control measures for STEC may potentially be applied in the production of raw beef. Some of the control measures of this document may be subject to approval by competent authorities.

11. While control in the primary production phase can decrease the number of animals carrying and/or shedding STEC, controls after primary production are important to prevent the contamination and cross-contamination of carcasses and, in particular, raw ground/minced beef. The systematic approach to the identification and evaluation of potential control measures allows consideration of the use of controls in the food chain and allows the application of control measures individually or in combination. This is particularly important as individual countries use different primary production and processing systems. Risk managers need the flexibility to choose risk management options that are appropriate to their national context.

12. STEC have a wide range of potential hosts, and STEC cells can potentially persist for over a year in the natural environment; therefore, control strategies based on preventing STEC infection of cattle or contamination of their environment would be difficult to implement in a reliable manner.

13. Interventions to control enteric pathogens should always be part of an integrated food safety system that includes all the stages from primary production to consumption. Measures to reduce STEC shedding or hide contamination prior to slaughter have the potential to reduce environmental exposure to STEC and may improve raw beef safety, but they cannot prevent STEC contamination or compensate for poor hygienic practices during slaughter, processing and distribution. Conversely, there is evidence that the adoption of good hygienic practices during slaughter and processing can minimise carcass contamination with STEC. Consequently, the adoption of best practices for preharvest management of cattle should be promoted as a support to hygienic slaughter and processing.

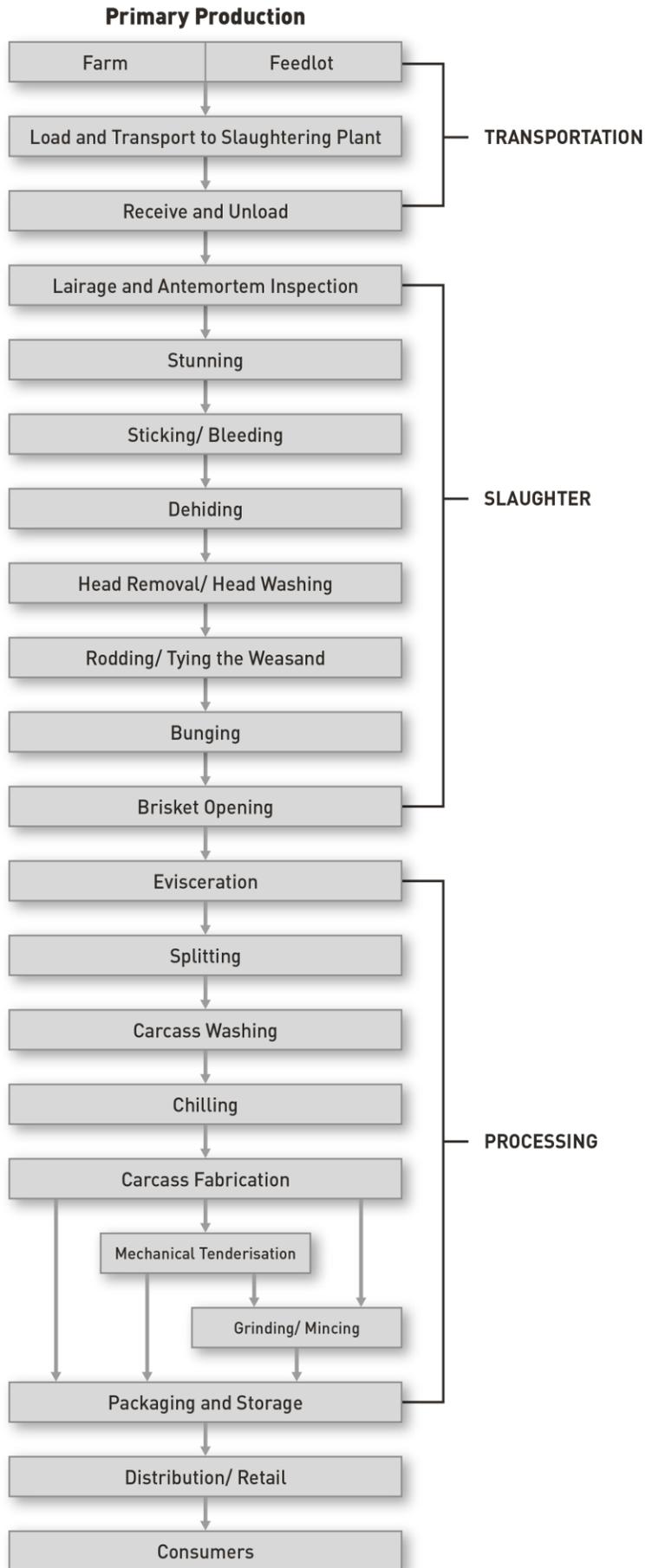
14. Operations to decontaminate carcasses or raw beef cuts will be of limited effectiveness if poor hygienic practices during subsequent processing and distribution permit recontamination or if the initial contamination load is high. Decontamination only reduces STEC by a certain amount, which can be quite variable depending on the type of treatment, duration, application, temperature, etc.

4.1 GENERIC FLOW DIAGRAM FOR APPLICATION OF CONTROL MEASURES

Process Flow Diagram: Primary Production-to-Consumption of Beef

15. These process steps are generic, and not all the steps may occur during processing, in the order shown, or at the same establishment. Grinding/mincing, for example, can be done at sites other than the slaughter or fabrication site. This flow diagram is for illustrative purposes only. For application of control measures in a specific country or an establishment, a complete and comprehensive flow diagram should be drawn up for each situation.

²¹ Tenderizing processes that include the injection of solutions with or without a vacuum are out of scope



4.2 PRIMARY PRODUCTION

16. Control measures to reduce the carriage of STEC in cattle prior to slaughter and that have the potential to reduce the prevalence of STEC are described in this section.

4.2.1 Specific Control Measures for Primary Production

17. The prevalence of STEC shedding in a herd and the individual animal shedding status for STEC is generally unpredictable, although factors have been identified that may influence STEC shedding. Interventions proposed to reduce the prevalence of STEC shedding or numbers of STEC shed by cattle include animal vaccination, dietary additives used in water and feeds, manipulation of animal feeds, and primary production management practices, as explained below.
18. Many of these proposed pre-slaughter control methods have not been demonstrated to reliably reduce the prevalence or the level of STEC shedding from cattle in a commercial setting. Research into pre-harvest control of STEC in cattle has focused on the serotypes O157:H7 and O157: NM and so there is often limited data available on the impact on other STEC serotypes. Additionally, some of the proposed methods are focused on specific subpopulations of STEC (e.g., vaccines, bacteriophage).

4.2.1.1 Diet Components

19. A wide variety of cattle diets have been investigated for their impact on STEC O157:H7 prevalence and/or level of shedding, including hay, barley, distillers and brewers' grains, sage brush, millet, alfalfa, (Callaway et al., 2009). Both STEC serotype O157:H7 and generic *E. coli* populations have been demonstrated to respond to changes in diet, but replication of results indicating STEC serotype O157:H7 reduction has been poor, and no dietary composition has been identified that reliably reduces STEC O157:H7. Some diets that have been proposed increase STEC serotype O157:H7 shedding (Thomas and Elliott, 2013).
20. In general, research supports that cattle on grain-based diets appear to shed higher levels of generic *E. coli* in their faeces than cattle on forage diets (Callaway et al 2003), but the effect of forage diets on faecal shedding of STEC serotype O157:H7 is inconclusive.

Use of Direct-Fed Microbials

21. [Use of probiotics or direct-fed microbials, involves feeding animals with viable microorganisms which are antagonistic toward pathogens, either by modifying environmental factors in the gut or producing antimicrobial compounds. There is evidence that specific direct-fed microbial treatments, such as *Lactobacillus acidophilus* (NP51) and *Propionibacterium freudenreichii* (NP24), can reduce STEC serotype O157:H7 shedding by cattle (Wisener et al., 2015, Venegas-Vargas et al 2016). The probiotics used should not contain antimicrobial resistance genes.]

Use of other feed additives

22. The seaweed *Ascophyllum nodosum* is marketed as a supplement for cattle feed. It has been reported to reduce faecal and hide prevalence of STEC serotype O157:H7 when added to corn feed (Braden et al., 2004).

4.2.1.2 Vaccination

23. Various vaccines have been designed and tested for preventing colonisation and/or reducing faecal shedding of STEC serotype O157:H7. Some vaccines have been shown to reduce faecal shedding of STEC serotype O157:H7 but their efficacy is dependent on the type of vaccine and the number of doses administered. Only a few vaccines have been tested under production conditions, and the duration of immunity after vaccination is unknown because the evaluation period in feedlot studies has been relatively short. The use of vaccination in cattle has not been commercially adopted due to the lack of evidence to support the reduction of STEC in beef following vaccination and the lack of farm-level incentives to cover additional costs associated with vaccines and their administration (JEMRA, 2020).

4.2.1.3 Good management practices at primary production

24. The following good management practices for cattle are recommended for minimising STEC shedding and hide contamination on animals presented for slaughter. Of particular concern is preventing the formation of faecal accumulation on animal hides, as this can interfere with hygienic dehiding and evisceration.

- Stressful situations should be minimized wherever possible, because increased stress increases shedding of pathogens (e.g., poor animal husbandry, rough handling, dietary stress and feed deprivation (Stein and Katz, 2017; Venegas-Vargas et al 2016)).
- Minimize exposure between herds to avoid or reduce horizontal transmission of STEC across herds (Callaway et al 2009).
- Maximize living space to reduce direct animal-to-animal transmission (e.g. maintain ample space for animals to move to reduce defecation directly onto one another).
- To the extent of possible, maintain clean living conditions (e.g., clean holding areas, remove gross contamination, and maintain clean and dry bedding) to prevent potential transmission from the living environment (e.g. animals resting in STEC-contaminated materials).
- Reduce the potential for STEC transmission through consumption of contaminated feed and water by the following:
 - Design feed and water delivery systems (tanks, trough, bins, etc.) in a way to reduce the potential for animal entrance and defecation.
 - Ensure water is of a microbiological quality that minimises animal contamination and, if there is doubt, treat the water.
 - Clean water troughs frequently to reduce replication and/or survival of STEC (Lejeune et al 2001).
 - Use materials in water troughs that facilitate the cleaning process; when possible, use metal troughs rather than troughs manufactured from concrete or plastic (Lejeune, 2001), which may chip or crack, creating areas for the bacteria to hide in and that are difficult to clean.

4.3 Transportation

4.3.1 Specific Control Measures for Transport to Slaughterhouse

25. Transportation can be a major contributor to the increasing occurrence of pathogens in cattle and a source of hide contamination. Contributing factors include mixing of animals of different origin, increased stress, increased exposure to STEC during extended duration of transportation, and cleanliness of transport vehicles (Norrung et al., 2008; Dewell et al. 2008, Stein and Katz, 2017).
26. Transportation practices should minimize any condition that could affect contamination of the meat. Control measures implemented prior to transportation may include:
 - Handle animals so that they are not unduly stressed.
 - Transport animals from the same herd in the same truck where possible to avoid social stress.
 - As much as practical, minimize the distance over which cattle are transported to slaughter; longer transportation distances have been shown to increase the risk of having STEC-positive hides at slaughter compared to cattle that travel a shorter distance (Dewell et al, 2008).
 - Ensure animals are as clean as possible to decrease the opportunity for pathogen contamination onto carcasses or hides during the slaughter and dressing processes. The likelihood of STEC contaminating the meat increases where levels of faecal contamination on the hide are high.
 - Load the animals onto clean vehicles, prevent faecal transfer from the top level to bottom level in multi-level trailers to the extent possible, and do not overcrowd the vehicle.
27. Cross-contamination among animals from different farms during transportation to the slaughter facility and at lairage (holding pens) can be an important source of hide contamination. Therefore, appropriate controls should be in place to minimize hide contamination. Controls may include:
 - Improve truck design, allowing for separation of animal lots.
 - When possible, separate lots of animals from different farms, use holding pens of an appropriate size for the number of animals, avoid overpopulation and stress of the animals.
 - Appropriately clean holding pens between lots of cattle.
 - Implement visual inspection and controls, when needed, for soiled animals, transportation vehicles and lairage pens for visible faecal contamination.

4.3.2 Specific Control Measures at Receive and Unload

28. Maintain herd integrity during load assembly and transport through unloading and placing in holding pens. To minimize STEC shedding, stress levels should be minimized using good animal handling practices; minimize or eliminate the use of electric prods and avoid overcrowding.
29. Adequate training of the operators on procedures that can minimize stress at this step (which could increase shedding of STEC) is recommended.

4.4 SLAUGHTER AND DRESSING

30. Interventions during primary processing (slaughter and dressing) at the slaughterhouse include physical, chemical, or biological interventions that can be applied alone or in combination; these are likely to reduce the number of STEC microorganisms but should not be considered to eliminate STEC on every carcass. Good hygiene practices (GHP) and emphasis on good manufacturing practices (GMP) at slaughter are necessary to prevent transfer of STEC from the hide and digestive tract to the carcass. Particular focus should be given to ensuring best practice in the operations of dehiding, head removal, clipping the weasand, bunging and evisceration, as these operations are the initial sources of microbiota that contaminate meat surfaces (Gill and Gill, 2010).
31. The specific control measures during this stage are intervention techniques aimed at preventing transfer of contamination to the carcass, as well as cross-contamination to other carcasses. Interventions selected should be validated for their effectiveness.
32. Interventions aimed at removing STEC from the surface of beef carcasses should consider that tolerance to salt and acid has been observed in some STEC strains. Determining the effectiveness of interventions to reduce microbial pathogens is complex, particularly as multiple interventions may be applied simultaneously or in sequence. The impact of interventions should be quantified by conducting experimental trials with surrogate microorganisms that have similar or greater resistance to individual treatments than STEC. Careful consideration is needed when determining suitable strains for validation of interventions, since surrogates may not necessarily be equivalent to wild-type strains isolated from raw beef.
33. Interventions should be safe and application feasible along the production process and should not change the organoleptic properties of beef.
34. The interventions described for the following steps may reduce the level of microbiota, including STEC, on raw beef. Many operations can be performed manually or with automated equipment. Automation of interventions offers the advantage of greater consistency of application but needs proper adjustment (Signorini et al., 2018).
35. Operators should be effectively and appropriately trained to perform their operation in the slaughter process in order to minimize the potential for STEC contamination.

4.4.1 Specific Control Measures at Lairage and Antemortem Inspection

36. In this stage the condition of the animals should be evaluated; animals should be as clean as possible to minimize the initial load count of microorganisms, which potentially includes STEC, on their hide. STEC is harboured on the hide not only in faecal material but also in dried-on dust. The level of both on the hide should therefore be minimized. Where practical, dirty, or wet animals should be segregated to prevent cross-contamination.
37. The lairage area should be cleaned as much as possible for each lot of animals using clean water under appropriate pressure to remove gross contamination on the floor. Cleaning and disinfection should be applied according to good hygiene practices and manufacturer's instructions. The lairage area should be designed to be well-drained in order to facilitate drying.
38. Practices such as washing animals (e.g., spray, mist, rinse, or wash), specifically the animal's hide, with different substances (e.g., clean water, bacteriophage) to reduce contamination has been investigated. However, in general, the evidence for washing in reducing the transfer of STEC from hide to carcass is low.
39. When feasible, at lairage cattle should not be comingled with other herds/lots to reduce social stress and prevent cross-contamination between herds/lots.

4.4.2 Specific Control Measures at Stunning, Sticking and Bleeding

40. Prior to stunning, animals may be sprayed in the accessway using low volume water jets at appropriate pressure. Similarly, the perianal region may be spray washed, but sparingly and only to remove feces (the source of STEC) released during the stunning process. Use of any water or rinses should be designed to reduce fecal and STEC contamination and not stress the animal or inhibit the stunning, sticking or bleeding effectiveness. Where water is applied, consideration should be given to removal of excess water prior to hanging of the carcass.
41. The stunning box and sticking table should be kept as clean as possible to avoid contamination of the animal's hide in the fall after the stunning process.
42. The stunning method employed (e.g., self-contained bolt, firearm, electrical stunning) can have different effects on STEC transfer into the skull.
43. In slaughter, special attention should be paid to avoid a delay in tying the weasand to minimize contamination of neck meat with STEC.
44. Sticking and bleeding should be done in a manner to reduce transfer of hide contamination to the carcass. Preparing the penetration or cut sites (e.g., with steam/vacuum treatment) can reduce the likelihood of contamination.
45. Allow an adequate distance between carcasses (i.e., avoid carcass-to-carcass contact), walls and equipment to minimise cross contamination during processing.

4.4.3 Specific Control Measures at Dehiding

46. Dehiding is the systematic process for separating the hide from the carcass and is perhaps one of the most critical operations in determining the level of STEC transferred to the carcass. To prevent transfer of contamination from the hide to the freshly exposed carcass, operators working at this stage should be appropriately trained to perform this operation to maximize hygienic dressing.
47. Slaughterhouses may consider, when feasible, a pre-hide removal carcass decontamination procedure to reduce hide contamination. Prior to dehiding, applying a process that decontaminates the hides (such as washes, hair removal, the application of bacteriophage cocktails or the application of steam and vacuum at the hide incision sites) may lower carcass microbial contamination. However, in general, the evidence on their role in reducing the transfer of STEC from hide to carcass is low. The excess liquid from the decontamination procedure should be removed (e.g., vacuumed) from the hide to avoid contamination of the carcass with liquid that could easily run onto the carcass when the hide is opened.
48. Rinsing of the rectum and disinfection of the perianal hide should be performed in order to reduce or eliminate contamination prior to dehiding. Hide-on carcass washes are frequently used for that purpose.
49. To prevent transfer of contamination from the hide to the carcass during hide opening (opening cuts), techniques can include:
 - Using clean and disinfected knives to cut through the hide.
 - Cleaning and disinfecting the knife (or tool) each instance the hide is penetrated, or using different knives, one to cut through the hide and the other to remove the hide.
 - Using a systematic trimming pattern, to work outward from a single hide opening site.
 - Using one hand to hold, pull and control the hide while separating/cutting the hide away from the carcass using the other hand.
 - Washing hands and aprons as often as needed to prevent cross-contamination of carcasses.
50. The number of workers and the role of their rotation in cross-contamination during the dehiding process needs to be considered and procedures used to prevent contamination.
51. The dehiding operation should be performed in a manner to avoid contact of the hide with the already exposed parts of carcass (i.e., dehiding the entire peri-anal region and bending the hide, making it stay above the tail). Using non-absorbent paper to protect specific areas of the carcass such as the brisket and bagging of the tail may also be useful practices for reduction of STEC contamination due to contact with hide during dehiding. Remove the hide from the top down rather from the bottom up to prevent contaminating the carcass with dust and hair that may be contaminated with STEC. Care should also be taken to avoid cross-contamination in

other operations carried out simultaneously with dehidating, such as the removal of the pizzle, the skinning of the shank tendons, the removal of the udder or scrotum, and transfers by overhead rail.

52. Measures should be taken to prevent tail flapping and contacting the carcass when hide pullers are used.

4.4.4 Specific Control Measures at Rodding

53. The rodding operation consists of using a metal rod to free the esophagus (weasand) from the trachea and surrounding tissues. In some countries, weasand meat may be recovered from the gastrointestinal tract for use in raw ground/minced beef production. The rodding operations should be performed in a manner to avoid contamination of the weasand and of the carcass interior from the exterior. If during the rodding operation the gastrointestinal tract is punctured, it can cause contamination of the carcass interior and exterior with ingesta.

54. To prevent cross-contamination of the carcass from the weasand/esophagus during the rodding operation, procedures can include:

- Hanging the carcass vertically, to cut the muscle and tissue to expose the esophagus.
- Using ties, clips, or bungs to close the weasand hygienically to prevent rumen spillage.
- “Dropping” heads by cutting the esophagus below the tie or clip.
- Changing or cleaning and disinfecting the weasand rod between each carcass.

55. If the gastrointestinal tract has been punctured, causing a major contamination, the carcass should be identified and additional procedures to avoid cross-contamination of other carcasses should be performed, such as separating the carcass immediately from the others.

56. When appropriately applied, these procedures will reduce contamination with gut microorganisms, but their specific effect on contamination by STEC remains unknown. Nevertheless, procedures that reduce faecal contamination are most likely to have an impact on STEC contamination.

4.4.5 Specific Control Measures at Bunging

57. Rectum occlusion should be performed hygienically in order to avoid contamination of the carcass and tools with the gastrointestinal contents or the hide, if the dehidating was not already done.

58. The use of separate clean knives for dehidating and rectum removal is recommended to avoid cross contamination of the rest of the carcass.

59. To prevent transfer of contamination from the bung to the carcass, techniques can include:

- Stuffing the bung with physical materials (e.g., paper towels) to push faecal material into the bung and reduce faecal movement out of the bung.
- Bag the bung by wrapping the bung in a bag and fastening it, i.e., with a rubber band, to contain any leakage that may occur during the evisceration process.

4.4.6 Specific Control Measures at Brisket Opening.

60. Brisket opening should be performed hygienically to avoid contamination of the carcass and tools, especially if dehidating has not been done.

61. To prevent introduction of contamination into the carcass during brisket opening, procedures can include:

- Cleaning and disinfecting the brisket saw and knife between each carcass and ensuring that the gastrointestinal tract is not punctured.
- If the gastrointestinal tract has been punctured causing a major contamination, the carcass should be identified and additional procedures to avoid cross-contamination of other carcasses should be performed, such as separating the carcass immediately from the others.

4.5 PROCESSING

62. STEC on the carcass can remain on meat cuts or be transferred to previously uncontaminated meat cuts as the carcass is further processed, especially via hands and meat processing equipment.

4.5.1 Specific Control Measures at Evisceration

63. Evisceration includes procedures to remove the digestive track and organs from the carcass. The evisceration should be done avoiding contamination with gastrointestinal contents due to a cut in the gastrointestinal tract.

64. To prevent contamination of the carcass by the viscera during removal, techniques can include the following:
- Removing visible contamination from the area to be cut (e.g., by trimming, by using air knives, or by steam vacuuming) before the cut is made. This should be done in a timely manner and in accordance with commonly accepted reconditioning procedures.
 - If the animal is pregnant, removing the uterus in a manner that prevents contamination of the carcass and viscera.
 - Avoid cutting through tonsils.
65. To prevent contamination of the carcass by employees during evisceration, techniques can include:
- The appropriate use of knives and equipment to prevent damage (i.e., puncturing) to the rumen and intestines.
 - Using footbaths or separate footwear by employees on moving from evisceration lines to prevent contaminating other parts of the operation.
 - Using trained and experienced individuals to perform the evisceration; this is particularly important at higher line speeds.
66. If the gastrointestinal tract has been punctured causing a major contamination, no further work should be carried out on the carcass until it has been removed from the slaughter line. Cleaning of the environment as well as operator protective equipment and tools being used at the time of the contamination event should be undertaken as needed, to prevent cross-contamination of leading and trailing carcasses.

4.5.2 Specific Control Measures at Carcass Splitting

67. Carcass Splitting is the point in the process where carcasses are split vertically into two halves.
68. To prevent the split carcass from becoming contaminated, techniques can include:
- Removing visible carcass defects that may contaminate the saw or cleaver (e.g., faeces, milk, ingesta, abscesses) in a sanitary manner before splitting the carcass.
 - Cleaning to remove organic material and disinfecting the saws and knives between each carcass.
 - Allowing adequate distance between split half carcasses and between different carcasses (i.e., avoid carcass-to-carcass contact), walls and equipment.
69. Targeted removal of visible contamination on carcasses by trimming may be applied to carcasses, but the disadvantage of trimming is potential cross-contamination from dirty knives (if not using a knife-switching disinfection protocol in-between cuts), aprons, mesh gloves, and waste. Also, even though practices may be effective at removing visible defects, the effectiveness of these practices to reduce pathogen contamination, including STEC, is limited.
70. Carcass trimming should be done in an area designated for that purpose and should result in trimmed carcasses that are free of stick wounds, blood clots, bruised tissue, pathological defects, visible contaminants, and dressing defects.

4.5.3 Specific Control Measures at Carcass Washing/Treatment

71. Carcass washing may remove visible soiling and reduce overall bacterial counts on beef carcasses by up to 1 log unit.

Carcass washing with antimicrobial agents.

72. Carcass washing with antimicrobial agents, such as organic acids (e.g., citric acid, lactic acid, acetic acid), oxidising agents (e.g., chlorine, peroxides, ozone) or other antimicrobial agents, in accordance with label directions, may be effective in reducing STEC. Such antimicrobial treatments may be applied with hot water to have a combined thermal impact. Factors determining the effectiveness of such treatments include the concentration of the agent, uniformity of surface coverage, the temperature of the solution, and the contact period. Individual STEC strains may vary in their sensitivity to such treatments. Organic acids alone can reduce but not completely eliminate STEC serotype O157:H7.

Carcass surface pasteurization.

73. This form of treatment is most commonly applied to carcass sides at the end of dressing. Water at >85 °C may be applied as a spray, a sheet or as steam (Gill and Bryant, 2000; Retzlaff et al., 2005). Treatment is most effective when applied to clean, dry carcass sides as large drops or sheets of water; when applied under such

conditions the treatment can achieve >2 log reductions in total *E. coli* in commercial slaughter operations (Gill and Jones, 2006).

Steam and vacuum

74. The carcasses are sprayed with steam and then an aspiration is performed, which fulfils a double function of eliminating and / or inactivating surface contamination. The manual device includes a vacuum tube with a hot water spray nozzle, which delivers water at approximately 82-88 ° C on the surface of the carcass. The process is effective in removing visible contamination in the carcasses.

4.5.4 Specific Control Measures at Chilling

75. Rapid chilling minimizes the potential for bacterial growth; STEC can only replicate at temperatures of 7 °C and above. The potential for bacterial growth is also dependent upon the water activity at the carcass surface, and if water activity is low enough (less than a_w 0.95), a decline in bacterial numbers will occur. Thus, controlling the humidity of the chilling process can impact STEC levels on the carcass. Alternatively, spray chilling with antimicrobial agents may reduce STEC survival.

4.5.5 Specific Control Measures at Mechanical Tenderization, Grinding/Mincing

76. Manufacturers should ensure that mechanical tenderizers and associated processing equipment are cleaned and disinfected on a regular basis to minimize the potential for translocating STEC from the exterior surface of the product to the interior and to minimize the potential for cross-contamination among lots of production. Manufacturers should also consider purchase specifications that require that incoming beef to be tenderised has been treated to eliminate or reduce STEC to an undetectable level or should apply such treatments prior to mechanical tenderization.

77. Antimicrobial washes, such as lactic acid, peroxyacetic acid and acidified sodium chlorite have been shown to reduce the concentration of *E. coli* serotype O157:H7 and other STEC on beef (i.e., carcasses, primal cuts, or other cuts) and could be used to minimize contamination of materials used to manufacture ground/minced beef.

78. To minimize STEC contamination and/or the spread contamination of ground/minced beef with STEC, measures may include, where appropriate:

- Storing products to prevent the growth of STEC. Multiplication of STEC is inhibited below 7°C, but low temperatures do not significantly reduce STEC. Establishments need to control STEC, using adequate time/temperature combinations.
- Cleaning equipment and the environment on a regular basis and ensuring employees follow good hygiene practices to avoid contamination.
- Specifying that all beef which will be used for grinding or already minced beef be pretested and found negative for specific strains of STEC, e.g., *E. coli* serotype O157:H7.
- Treating the outer surfaces of the meat with organic acid sprays or other approved treatments before grinding/mincing.
- Appropriately chilling raw meat during production to reduce possible multiplication of STEC if they are present.

79. Since processes such as grinding/mincing may potentially spread contamination in the meat, there should be increased awareness when handling ground/minced beef products throughout the rest of the food chain.

4.5.6 Specific Control Measures at Packaging and Storage

80. A range of non-thermal preservation technologies (e.g., pulsed light, natural bio-preservatives, high hydrostatic pressure, ionizing radiation) and thermal preservation technologies (e.g., microwave and radiofrequency tunnels, Ohmic heating or steam pasteurization) have been investigated for meat decontamination either during processing or after final packaging. The practical use of these methods is dependent upon the impact on the organoleptic properties of the meat and its final use. Factors determining the effectiveness of such treatments include the sensitivity of the microorganism, the temperature of the environment, the intrinsic characteristics of the food (e.g., fat content, salt, additives, pH) and the level of initial contamination.

81. During packaging and storage, the time/temperature combination should be such that one generation of bacterial growth cannot occur.

4.6. DISTRIBUTION/ RETAIL

4.6.1 *Specific Control Measures at Distribution and Retail*

82. Control of refrigeration temperatures should be maintained during transport and storage of the carcasses, beef cuts, or minced/ground beef along the distribution chain until the product reaches the consumer.
83. If product is removed from the original package for further processing or re-portioning, appropriate good hygienic practices should be observed to avoid recontamination with STEC.

Packaging conditions

84. Ground/minced products should have sufficient information so that the recipient can safely handle and prepare the product e.g., use-by dates and the need for thorough cooking on the label.
85. Since not all tenderized products are readily distinguishable from non-tenderized products, labelling to state that the product is tenderized, along with validated cooking instructions, should be included to provide consumers and food service workers the essential information to safely prepare the product.

4.7. CONSUMERS

86. The consumer has an important role in the prevention of foodborne illness from STEC during the manipulation of raw beef at home and should be made aware of the proper cooking and handling of raw beef.
87. Since “non-intact” raw beef products may pose an increased risk for consumers, appropriate consumer guidance on safe handling, including cooking temperatures, may be needed.
88. Consumers should apply the general principles for safer food to ensure safety of raw beef when handling, preparing and consuming beef; these are.
 - Keep the food preparation and consuming sites clean,
 - Separate raw and cooked food to avoid/prevent cross-contamination.
 - Cook appropriately.
 - Keep food at safe temperatures.
 - Use safe water and raw materials for food preparations.

5. **VALIDATION OF CONTROL MEASURES**

89. Refer to the general section of this guidance.

6. **MONITORING OF CONTROL MEASURES**

90. Monitoring data are used to measure the effectiveness of any control measure put in place, to establish alternative or improved measures, and to identify trends and emerging STEC hazards, food vehicles, and food chain practices.
91. Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring indicator microorganisms. These indicator microorganisms do not indicate pathogen presence; instead, they provide a quantitative measure of the control of microbial contamination in the product and processing environment. Periodic testing for the STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country) may also be conducted for verification of process performance.
92. Some raw beef will need more control measures and monitoring than others (e.g., non-intact raw beef).

7. **VERIFICATION OF CONTROL MEASURES AND REVIEW OF CONTROL MEASURES**

93. STEC testing is an important part of verification of process performance. However, STEC are generally present at very low levels and are characterised by heterogeneous distribution (including in ground/minced products), making STEC detection challenging. This means that there may be a significant delay in detecting loss of process control based on STEC detection. Consequently, verification programs should also include quantitative monitoring of indicator microorganisms. Hygiene indicators used should be those that are the most informative for the specific processing environment. An increase in the numbers of the selected indicator microorganisms indicates decreasing process control and corrective action should be taken. The speed in detecting a loss of control increases with the verification frequency. Verification at multiple points in the

processing chain can assist in rapid identification of the specific process where corrective action should be taken.

94. Regular testing for STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country) can also be conducted for verification of process performance. Lot testing is of significant utility, particularly in raw beef that is intended for further processing into ground/minced beef and contributes to directly reducing contamination rates in retail ground/minced beef and promoting continuous process improvement.
95. Verification of other control measures, e.g., concentration of organic acid, temperature of a steam/vacuum or hot water treatment, etc., should be routinely conducted in addition to appropriate microbiological testing.

8. CONSIDERATIONS FOR LABORATORY TESTING FOR DETECTION OF STEC IN RAW BEEF

96. Intact raw beef cuts used for purposes other than the manufacture of finished ground or blade tenderized raw beef products do not present the same level of risk, since STEC will be on the external surfaces that will receive the most heat in cooking; testing for STEC therefore offers little value. However, when the final intended use of raw beef cuts is not known, sampling should be implemented for STEC strains considered to be a country's highest priority verification.
97. In general, the occurrence of STEC in meat products is lower for intact meat products than in trim or ground / minced beef. However, the overall occurrence of STEC in these products can vary considerably due to differences in primary processing and post-processing conditions and interventions.
98. Levels of STEC in non-intact raw beef, such as ground/ minced products, are often higher than in intact beef because ground or disrupted tissue presents an environment that is more conducive for bacterial growth. In addition, many of the processing and post-processing interventions are more efficacious if the targeted pathogen is exposed on the surface of the meat as opposed to embedded within a tissue matrix.
99. Trim and ground/minced raw beef can originate from the tissues of multiple carcasses, whereas an intact raw beef product would be from a single carcass. The process of amalgamation of tissues from multiple animals/herds can increase the risk of contamination of ground / minced raw beef, therefore more testing should be conducted.

FRESH LEAFY VEGETABLES

INTRODUCTION

1. Fresh leafy vegetables are grown, processed and consumed throughout the world. They are grown on farms of varying sizes; distributed and marketed locally and globally, providing year-round availability to consumers; and sold as fresh, fresh pre-cut or other ready-to-eat (RTE) products such as pre-packaged salads.
2. Outbreaks of illness caused by a broad range of microbial pathogens, including Shiga toxin-producing *Escherichia coli* (STEC), have been linked to the consumption of fresh leafy vegetables. Epidemiological evidence, outbreak investigations, research, and risk assessments have identified several possible contamination sources of fresh leafy vegetables with STEC, including water, domestic and wild animals, workers and manure-based soil amendments²². Fresh leafy vegetables are typically grown and harvested in large volumes, increasingly in places where harvest and distribution of fresh leafy vegetables is efficient and rapid. Fresh leafy vegetables are packed in diverse ways, including: field packed direct for market; field cored and prepared for later processing; and as pre-cut fresh leafy vegetable mixtures and blends with other vegetables. Control measures such as antimicrobial washes to minimize cross-contamination may be applied prior to packaging and/or shipment to market. As fresh leafy vegetables move through the supply chain, there is also the potential for the introduction and growth of pathogens, including STEC. The increasing worldwide use of pre-packaged fresh-cut leafy vegetables to expand the supply chain might increase the potential for the presence of contaminated product in the marketplace through cross-contamination with STEC, and STEC replication during distribution and storage if fresh-cut leafy vegetables are improperly handled. There is no processing treatment applied that would eliminate or inactivate STEC, although contamination can be reduced by washing in water containing antimicrobials. Examples of field level control measures provided in this document are illustrative only and their use and approval may vary by country.
3. It is recognized that some of the provisions in this Annex may be difficult to implement in areas where primary production is conducted in smallholdings, whether in developed or developing countries, and in areas where traditional farming is practiced. The Annex is, therefore, a flexible one, to allow for diverse systems of control and prevention of contamination for different cultural practices and growing conditions. Figure 1 provides a flow diagram illustrating a generalized process flow for fresh leafy vegetables. This flow diagram is for illustrative purposes only. Steps may not occur in all operations (as shown with dotted lines) and may not occur in the order presented in the flow diagram.

1. OBJECTIVE

4. The objective of this Annex is to provide guidance to reduce, during production, harvesting, packing, processing, storage, distribution, marketing and consumer use, the risk of foodborne illness from STEC associated with fresh leafy vegetables intended for human consumption without cooking.

2. SCOPE AND DEFINITIONS

2.1 Scope

5. This Annex covers specific guidance for the control of STEC related to fresh leafy vegetables that are intended to be consumed without cooking. The Annex is applicable to fresh leafy vegetables grown in open fields or in fully or partially protected facilities (hydroponic systems, greenhouses/controlled environments, tunnels etc.).

2.2 Definitions

6. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), including Annex I for Ready-to-Eat Fresh, Pre-cut Fruits and Vegetables and Scope of Annex III for Fresh Leafy Vegetables.

Fresh leafy vegetables - Vegetables of a leafy nature where the leaf is intended for consumption without cooking, including, but not limited to, all varieties of lettuce, spinach, cabbage, chicory, endive, kale, radicchio, and fresh herbs such as coriander, cilantro, basil, curry leaf, colocasia leaves and parsley, among other local products for foliar consumption.

²² "Soil amendments" are fertilizers soil improvers, conditioners, or other material added to a soil to improve nutrients or the soil's physical properties, such as water retention, permeability, water infiltration, and drainage.

3. PRIMARY PRODUCTION

7. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003). As noted in CXC 1-1969, some of the principles of HACCP can be applied at primary production and may be incorporated into Good Agricultural Practices for the production of fresh leafy vegetables to minimize contamination with STEC.

8. Most contamination of fresh leafy vegetables with STEC is thought to occur during primary production. Fresh leafy vegetables are grown and harvested under a diverse range of climatic and geographical conditions. They can be grown in production sites indoors (e.g., greenhouses) and outdoors, harvested, and either field-packed or transported to a packing establishment, using various agricultural inputs and technologies, and on farms of varying sizes. In each primary production area, it is necessary to consider the agricultural practices and procedures that could minimize the potential for contamination of fresh leafy vegetables with STEC, taking into account the conditions specific to the primary production area, type of products, and growing (including irrigating) and harvesting methods used.

3.1 Environmental Conditions

9. Potential sources of STEC contamination should be identified prior to primary production activities and periodically evaluated for changes. Where possible, growers should evaluate present and previous uses of both indoor and outdoor fresh leafy vegetable primary production sites and the nearby and adjacent land (e.g., animal production, sewage treatment site) in order to identify potential sources of STEC. The assessment of environmental conditions is particularly important because subsequent interventions would not be sufficient to fully remove STEC contamination that occurs during primary production, and in some cases, conditions may enable the growth of STEC, thereby increasing the risk of illness for consumers.

10. If the environment presents a likelihood of contamination of the primary production site with STEC, measures should be implemented to minimize the potential for contamination of fresh leafy vegetables at the site. [Once product is contaminated with STEC it is not possible to eliminate it and there are limited control measures that can be implemented to reduce it.] When the likelihood of contamination cannot be managed or minimized, the production site should not be used for fresh leafy vegetable production.

11. The effects of some environmental events cannot be controlled and may need to be evaluated. For example, heavy rains or flood events may increase the exposure of fresh leafy vegetables to STEC if soil contaminated with STEC splashes onto them. When heavy rains occur, growers should evaluate the need to postpone harvesting fresh leafy vegetables for consumption without cooking and/or to subject them to a treatment that will minimize consumer exposure to STEC. If fresh leafy vegetables that contact flood waters are not subjected to any measure to mitigate risks from STEC to consumers, they should not be consumed raw. This does not include flooding of furrows for irrigation purposes, where the source of water is known and appropriate quality and is not the result of a weather event.

3.1.1 Location of the Production Site

12. Animal production facilities located in proximity to sites where fresh leafy vegetables are grown and access to the growing site by wildlife can pose a significant likelihood of contamination of production fields or water sources with STEC. Concentrated animal feeding operations and cattle grazing lands present a significant risk of contamination of leafy greens in the field; although guidelines exist for the distance between fields and nearby animal operations, the safe distance depends on factors that can increase or decrease the risk of contamination, such as topography of the land and opportunity for water runoff through or from such operations. Growers should evaluate the potential for such contamination and take measures to mitigate the risk of STEC contamination associated with runoff and flooding (e.g., terracing, digging a shallow ditch to prevent runoff from entering the field).

3.1.2 Animal activity

13. Some wild and domestic animals present in the primary production environment are known to be potential carriers of STEC. Wild animals represent a particularly difficult risk to manage because their presence is intermittent. The following are particularly important to minimize the potential for animal contamination of fresh leafy vegetables with STEC:

- Appropriate methods should be used in order to exclude animals from the primary production and handling areas to the extent practicable. Possible methods include the use of physical barriers (e.g., fences) and active deterrents (e.g., noise makers, scarecrows, images of owls, foil strips).

- Primary production and handling areas should be properly designed and maintained to reduce the likelihood of attracting animals that can contaminate fresh leafy vegetables with STEC. Possible methods include minimizing standing water in fields, restricting animal access to water sources, and maintaining production sites and handling areas free of waste and clutter.
- Fresh leafy vegetable primary production areas should be regularly checked for evidence of the presence of wildlife or domestic animal activity (e.g., presence of animal faeces, bird nests, hairs/fur, large areas of animal tracks, burrowing, decomposing remains, crop damage from grazing), particularly near the time of harvesting. Where such evidence exists, growers should evaluate the risks to determine whether the fresh leafy vegetables in the affected area of the production site should be harvested for consumption without cooking.

3.2 Hygienic primary production of fresh leafy vegetables

3.2.1 Water for primary production

14. Several parameters may influence the likelihood of contamination of fresh leafy vegetables with STEC: the source of water used for irrigation and the application of fertilizers and pesticides, the type of irrigation (e.g. drip, sprinkler, overhead), whether the edible portions of fresh leafy vegetables have direct contact with irrigation or other water, the timing of irrigation in relation to harvesting and, most importantly, the occurrence of STEC in the water used for irrigation or application of pesticides or fertilizers. Growers should evaluate the sources of water used on the farm for the likelihood of contamination with STEC and identify corrective actions to prevent or minimize STEC contamination (e.g., from livestock, wildlife, sewage treatment, human habitation, manure, and composting operations, or other intermittent or temporary environmental contamination, such as heavy rain or flooding). (Refer to section 3.2.1.1 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).)

15. [Growers should periodically test the water they use for appropriate indicator microorganisms and, where necessary, STEC,] according to the risk associated with the production. The frequency of testing will depend on the water source (i.e., lower for adequately maintained deep wells, higher for surface waters), the risks of environmental contamination, including intermittent or temporary contamination (e.g., heavy rain, flooding), or the implementation of a new water treatment process by growers. If the intended water source is found to contain unacceptable levels of indicator microorganisms or is contaminated with STEC, corrective actions should be taken to ensure that the water is suitable for its intended use. Possible corrective actions to prevent or minimize contamination of water for primary production may include the installation of fencing to prevent large animal contact, the proper maintenance of wells, water filtering, chemical water treatment, the prevention of the stirring of the sediment when drawing water, the construction of settling or holding ponds or water treatment facilities. The effectiveness of corrective actions should be verified by periodic water testing. Where possible, growers should have a contingency plan in place that identifies an alternative source of water fit for purpose.

16. It is especially critical in hydroponic operations to maintain the quality of water used as a growth medium for fresh leafy vegetables to reduce the likelihood of contamination and survival of STEC; the nutrient solution used may enhance the survival or growth of STEC. (Refer to section 3.2.1.1.3 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).)

3.2.2 Manure, biosolids and other natural fertilizers

17. The use of manure, biosolids and other natural fertilizers in the production of fresh leafy vegetables should be managed to limit the potential for contamination with STEC, which can persist in manure, biosolids and other natural fertilizers for weeks or even months, if the treatment of these materials is inadequate. Composting can be effective in controlling STEC in manure, depending on factors that include time, temperature, indigenous microorganisms, moisture, composition of the compost, pile size, and turning of the pile. Another manure treatment method involves anaerobic digestion. Treatment methods should be validated to inactivate STEC. Refer to section 3.2.1.2 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for practices to minimize microbial pathogens such as STEC in manure, biosolids and other natural fertilizers.

3.2.3 Personnel health, hygiene, and sanitary facilities

18. Hygiene and health requirements should be followed to ensure that personnel who come into direct contact with fresh leafy vegetables prior to, during or after harvesting will not contaminate them with STEC. Adequate access to, and use of, hygienic and sanitary facilities, including adequate means for hygienically washing and drying hands, are critical to minimize the potential for workers to contaminate fresh leafy vegetables. People known or suspected to be suffering from illness due to STEC should not be allowed to enter any area handling leafy

vegetables, including the harvest area. Refer to section 3.2.3 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for practices to minimize microbial pathogens such as STEC.

3.2.4 Harvesting

19. The field should be evaluated for animal intrusion, the presence of faecal deposits, or other sources of STEC contamination prior to harvest to determine if the field or portions thereof should not be harvested. Growers should avoid moving harvesting equipment across fields where manure or compost was applied. Harvesting equipment should be cleaned and disinfected as needed to avoid the contamination of fresh leafy vegetables (e.g., if the equipment runs over an area with animal intrusion and faecal deposits). Containers stored outside and field containers to be re-used should be cleaned and, as appropriate, disinfected before being used to transport fresh leafy vegetables.

3.2.5 Field packing

20. When packing fresh leafy vegetables in the field, care should be taken to avoid contaminating containers or bins by exposure to manure or other contamination sources. When fresh leafy vegetables are trimmed or cored in the field, knives and cutting edges should be cleaned and disinfected frequently to minimize the potential for cross-contamination with STEC.

3.2.6 Storage and transport from the field to the packing or processing facility

21. Fresh leafy vegetables should be stored and transported under conditions that will minimize the potential for STEC contamination and/or growth. Fresh leafy vegetables should not be transported in vehicles previously used to carry potentially contaminated materials, e.g., heavily soiled root vegetables, live animals, animal manure, compost, or biosolids. When vehicle receptacles or containers have been used for the transport of products other than fresh leafy vegetables, effective cleaning should be carried out between loads to avoid the risk of contamination.

4. PACKING OPERATIONS

22. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

4.1 Time and temperature control

23. Refer to the *General Principles of Food Hygiene* (CXC 1-1969). Time and temperature control during packing and storage is essential to prevent growth of any STEC that may be present, since an increase in numbers of STEC will increase the risk of illness.

4.2 Cooling fresh leafy vegetables

24. As far as possible, the cooling of fresh leafy vegetables should take place as rapidly as possible to minimize growth of any STEC that may be present and in a manner that does not contribute to contamination of product with STEC. For example, fresh leafy vegetables can be cooled immediately after harvest by using ice (e.g., for parsley), forced-air cooling, vacuum cooling (e.g., for iceberg lettuce), hydrocooling or spray-vacuum (hydro-vac) cooling.

25. If water used for cooling comes into direct contact with the fresh leafy vegetables, it should be controlled, monitored, and recorded to ensure that the concentration of biocides is sufficient to minimize the likelihood of cross-contamination.

4.3 Washing fresh leafy vegetables

26. Packers washing fresh leafy vegetables should follow good hygienic practices (GHPs) to prevent or minimize the potential for the introduction or spread of STEC in wash water. Where used, biocides should be added to wash water as per GHPs, with their levels monitored, controlled and recorded regularly during production to ensure the maintenance of effective concentrations. The characteristics of post-harvest water that may impact the efficacy of the biocidal treatments (e.g., the pH, turbidity and water hardness) should be controlled, monitored and recorded.

5. PROCESSING OPERATIONS

27. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), including Annex III on Fresh Leafy Vegetables and Annex I on Ready-to-Eat, Fresh, Pre-Cut Fruits and Vegetables.

28. It is recommended that unprocessed fresh leafy vegetable handling areas be physically separated from processing areas to minimize contamination with STEC. Processing, with some exceptions (e.g., cooking) cannot fully eliminate STEC contamination that may have occurred during primary production of fresh leafy vegetables. Processors should ensure that growers, harvesters, packers, and distributors have implemented measures to minimize the contamination during primary production of the fresh leafy vegetables and also during subsequent handling in accordance with the provisions in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

5.1 Time and temperature control

29. Refer to the *General Principles of Food Hygiene* (CXC 1-1969). Time and temperature control during pre-processing storage, processing and post-processing storage is essential to prevent growth of any STEC that may be present, since an increase in numbers will increase the risk of consumer illnesses. A temperature of 7°C or below will prevent growth of STEC and is appropriate for those fresh leafy vegetables that are not subject to cold injury.

5.2 Trimming, coring, cutting and shredding of fresh leafy vegetables

30. Cutting knives and other cutting tools, equipment and any other contact surfaces, should be cleaned and disinfected frequently to minimize the potential for transfer of STEC.

5.3 Washing and dewatering/drying cut fresh leafy vegetables

31. Washing and drying are important steps in the control of STEC for fresh-cut leafy vegetables. See Section 4.3 above and section 5.2.2.5.1 of Annex I on Ready-to-Eat, Fresh, Pre-Cut Fruits and Vegetables of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003),

5.4 Cold storage

32. When feasible Fresh leafy vegetables should be maintained at appropriate temperatures after cooling to minimize growth of any STEC that may be present. The temperature of the cold storage should be controlled, monitored and recorded.

5.5 Microbiological and other specifications

33. Microbiological testing of fresh leafy vegetables and of water for primary production for STEC is currently of limited use due to difficulty in detecting STEC because of low prevalence and low numbers of the organism in fresh leafy vegetables and in water. Testing of fresh leafy vegetables for indicator microorganisms, supplemented, where appropriate, by periodic testing for STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country), can be a useful tool to evaluate and verify the safety of the product and the effectiveness of the control measures and to provide information about an environment, a process or even a specific product lot when sampling plans and testing methodology are properly designed and performed. Measures to be undertaken in case of positive results for STEC (or when indicator microorganisms reach a pre-defined threshold) need to be established and defined. Refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).

5.6 Documentation and records

34. It is recommended that harvesting, processing, production, and distribution records should be retained long enough to facilitate STEC illness investigation and recalls if needed. This period may significantly exceed the shelf-life of fresh leafy vegetables. Refer to section 5.7 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for the types of records that should be maintained by growers, harvesters and packers that may be important when investigating foodborne illness outbreaks due to STEC.

6. ESTABLISHMENT: MAINTENANCE AND SANITATION

35. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

7. ESTABLISHMENT: PERSONAL HYGIENE

36. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

8. TRANSPORTATION

37. Refer to the *General Principles of Food Hygiene* (CXC 1-1969), the *Code of Hygienic Practice for the Transport of Food in Bulk and Semi-Packed Food* (CXC 47-2001) and the *Code of Practice for the Packaging and Transport of Fresh Fruits and Vegetables* (CXC 44-1995).

9. PRODUCT INFORMATION AND CONSUMER AWARENESS**9.1 Lot identification**

38. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

9.2 Product information

39. Refer to the *General Principles of Food Hygiene* (CXC 1-1969).

9.3 Labelling

40. Refer to the *General Standard for the Labelling of Pre-packaged Foods* (CXS 1-1985) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

9.4 Consumer education

41. Refer to the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

10. TRAINING

42. Refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

11. RETAIL AND FOODSERVICE

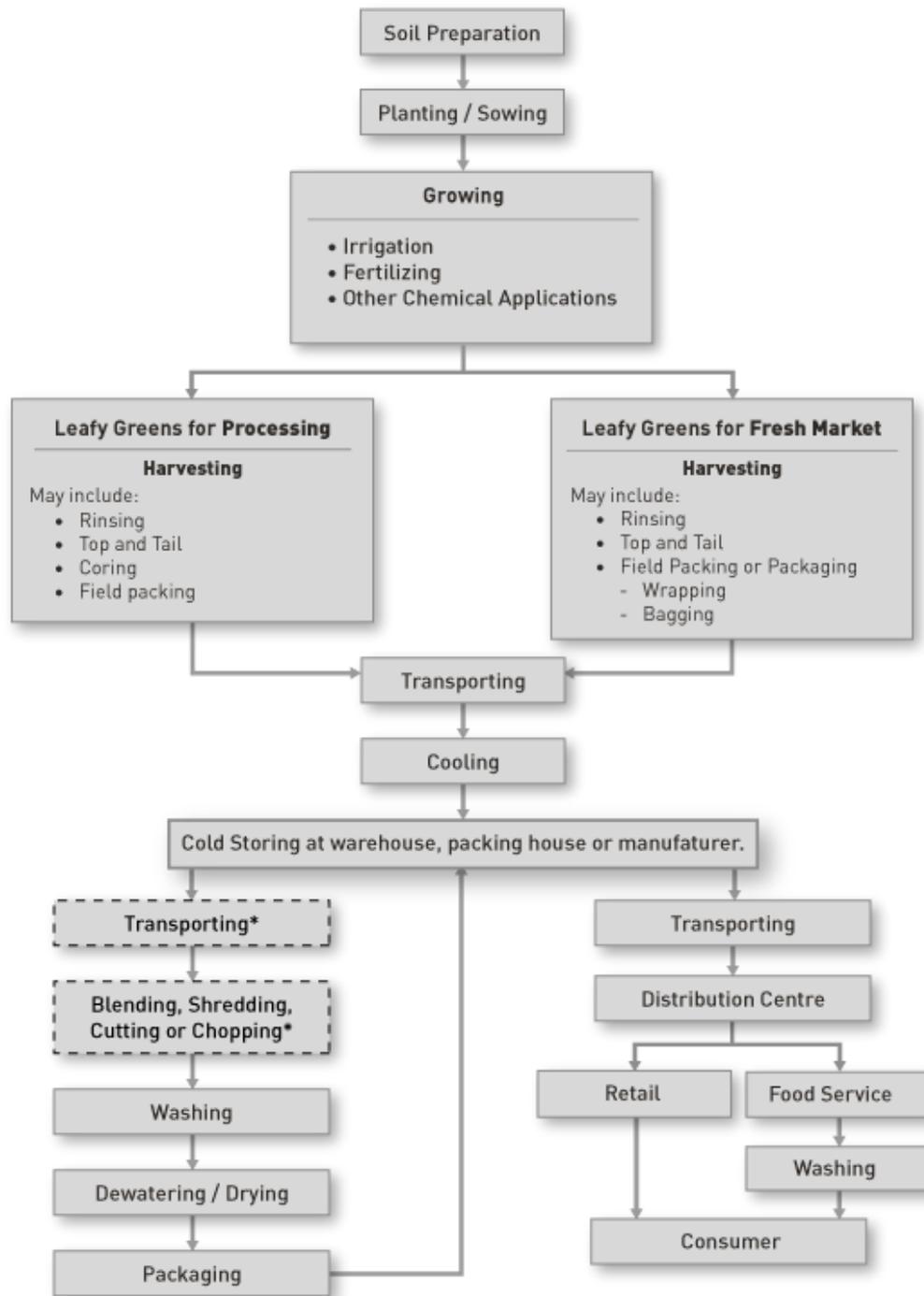
43. Fresh leafy vegetables (intact and pre-cut) should be held at an appropriate temperature to minimize growth of STEC. Cross-contamination from or to other food items should be prevented. Food business operators serving fresh leafy vegetables for consumption without cooking to consumers should take appropriate measures to

- prevent cross-contamination,
- maintain appropriate storage temperature,
- thoroughly wash fresh leafy vegetables prior to use, and
- ensure proper cleaning of tools and surfaces that may come in contact with these products.

12. CONSUMER

44. See section 9.4 in the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003).

Figure1: Fresh Leafy Vegetables Flow Diagram²³



²³ The diagram illustrates a generalised process flow for fresh leafy vegetables for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram.

*Boxes with broken lines indicate steps that may not be included, depending in part on the commodity.

RAW MILK AND RAW MILK CHEESES

1. INTRODUCTION

1. Although most milk for drinking is either pasteurized or sterilized by ultra-high temperature (UHT) processing, raw drinking milk is consumed in many countries. [Consuming raw drinking milk without any control measures is associated with a higher risk of illness]. Raw milk cheeses are fermented products made from raw milk that are consumed in a variety of countries around the world. [Without any control measures, they are associated with a higher risk of foodborne illness than those cheeses made from milk subject to heating such as thermization²⁴ or pasteurization to reduce the risk from foodborne pathogens]. Cheeses are produced by both large manufacturers and small factories such as farm cheese producers, artisanal cheese producers or large-scale industry and cheese makers. Specific combinations of ingredients and cheese-making processes are used by manufacturers to obtain a wide variety of cheeses with desired characteristics that meet consumer expectations.

2. Raw milk and raw milk cheeses have been associated with foodborne infections caused by Shiga toxin-producing *Escherichia coli* (STEC) in humans from different countries. The infectious dose for STEC in raw milk or raw milk cheese is low. A comprehensive approach, considering all the aspects of raw milk and raw milk cheeses from production to consumption, is necessary to reduce the presence of STEC in these products.

3. Cattle are a main source of STEC. Infected cattle can carry the bacteria in their gastrointestinal tract without any symptoms of disease and shed them in their faeces. STEC have also been isolated from the faeces of other species of animals, including buffaloes, goats, camels and sheep, that are commonly milked for human consumption. Detailed investigations have shown that without observance of appropriate cleaning and disinfecting steps and udder good hygiene practices, faecal matter can contaminate the cow's teats and udders, which can increase the risk of microbial contamination of the milk during the milking process. For this reason, STEC can potentially be found in raw milk. When STEC-contaminated milk is used to produce raw milk cheeses, STEC may survive and be isolated from some resulting raw milk cheeses.

4. Raw milk cheeses are made from raw milk coagulated through the action of rennet, selected microbiological organisms or other suitable coagulating agents, and then partially or completely draining the whey resulting from the coagulation, while adhering to the principle that cheese-making results in a concentration of milk protein and milk fat. Following this step, different processing techniques are applied to produce the end-products. Different microbiota and very diverse enzymatic reactions play a complex role during processing and maturation. This results in very different cheese types, including ripened or unripened soft, semi-soft, semi-hard, hard, or extra-hard product, which may be coated, uncooked, pressed, and sold fresh (unripened) or ripened. The different processing steps applied, and the raw milks used from different species (e.g., cow, buffalo, goat, sheep) can influence the behavior (survival, growth or inactivation) of STEC strains

5. This document is intended for use by a variety of food business operators (FBOs) using diverse milk production systems and cheese-making processes, therefore flexibility has been included throughout it to allow different systems of control and prevention of contamination considering cultural matters and different processing practices and conditions.

6. This guidance describes the surveillance and the good practices that can contribute to control STEC in raw milk and raw milk cheeses at different steps in the production chain and, when implemented correctly, can help reduce the risk of contamination and resulting illness. Effectiveness of interventions of different production practices to control STEC based on published data is variable. This is due to the significant differences in experimental design and manufacturing practice among studies. In particular, the efficacy of control measures at multiple steps in the food chain on the overall reduction of concentration of STEC in raw milk and raw milk cheeses has not been quantified. Consequently, it will be up to competent authorities and each operator (farmer, dairy, or FBO) to define and implement appropriate risk-based monitoring and control measures, considering relevant scientific and technical information.

²⁴ Thermization, a sub pasteurization heat treatment (55.0–71.7°C), has been proposed to reduce the risk of pathogens in raw cheese milk while retaining some quality attributes in the cheese.

2. OBJECTIVE

7. The objective of this annex is to provide science-based guidance for the control of STEC related to raw drinking milk and raw milk cheeses. This guidance focuses on control of STEC during raw milk production (cows, buffaloes, goats, camels and sheep), raw milk cheese making, storage, and distribution to consumers.

3. SCOPE AND DEFINITIONS

3.1. Scope

8. This annex presents specific guidance for control of STEC related to raw milk intended for drinking and for raw milk cheeses.

3.2. Definitions

- Refer to the *General Standard for the Use of Dairy Terms* (CXS 206-1999), and the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) Annex I (Guidelines for the Primary Production of Milk) and Annex II (Guidelines for the Management of Control Measures During and After Processing). Also refer to the *General Principles of Food Hygiene* (CXC 1-1969) and the *General Standard for Cheese* (CXS 283-1978).
- **Milk:** Milk is the normal mammary secretion of milking animals obtained from one or more milking's without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing²⁵
- **Raw milk:** Milk (as defined in the *General Standard for the Use of Dairy Terms* (CODEX STAN 206-1999)) which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect.^{26, 27, 28}
- **Raw milk cheeses:** Cheeses made from raw milk.

4. PRIMARY PRODUCTION-TO-CONSUMPTION APPROACH TO CONTROL MEASURES

9. Figures 1 and 2 provide flow diagrams describing key steps of raw milk and raw milk cheeses production. Not all steps occur in all operations, there may be other steps, and steps may occur in a different order than shown in the Figures.

10. Raw milk should come from healthy animals, be obtained by hygienic milking practices and be free of colostrum. Raw milk can be a potential source of microbial pathogens, including STEC. It is of major importance to ensure the sanitary quality of the raw milk, which does not undergo a microbial reduction treatment prior to packaging for drinking milk or before cheese making.

11. The application of combined control measures throughout the food chain particularly at the farm, transport and processing is necessary for the control of STEC in the end-products. However, these measures and flow diagrams can vary according to different dairy farming practices and cheese-making processes.

5. PRIMARY PRODUCTION – MILK PRODUCTION AT DAIRY FARM

5.1. Control measures for STEC for dairy herds at the dairy farm.

12. STEC are commonly present in the microbiota of milk-producing animals, and it is not possible to eradicate them. The excretion of STEC by ruminants seems to be sporadic but may also be persistent over several months. Studies have shown that excretion varies according to the season, peaking in warmer months. Excretion also varies among individual cows, with some individuals considered to be “high shedders” (a high-level excretion of STEC), and excretion levels may even differ between cow droppings of the same animal. Other factors proposed to contribute to changes in STEC excretion include age, diet, housing, stress, herd size, animal health, geographical area, and previous contamination with STEC strains. Faecal contamination of sheep and goat milks exist but is less likely than for cows, because of anatomical differences and as their faeces tend to be more solid and thus are less likely to cross-contaminate. There are no established methods to prevent STEC carriage or

²⁵ Codex *General Standard for the Use of Dairy Terms* (CXS 206-1999).

²⁶ The *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004).

²⁷ Heat treatment beyond 40°C results in changes such that the structure of the resultant product is no longer the same as that of raw milk. In addition, a point temperature of 40°C, and up to pasteurization temperatures, is generally considered to be insufficient to kill STEC in raw milk.

²⁸ Milk that has been subject to processing techniques such as microfiltration and/or bacto-fugation is no longer considered raw milk.

ensure reduced shedding by ruminants. In addition, no interventions specific for small ruminants are suggested. Control measures should be implemented to minimize spread between animals and their environments. The following are examples of measures that may be useful:

- maintain animal health and minimize animal stress,
- keep litter and bedding as dry as possible and remove them when they become soiled with excess manure.

13. Other wildlife or livestock, pests, and birds can also carry STEC and thus contribute to their circulation in milking herds. Applying comprehensive pest management may be useful.

14. Animal-to-animal transmission via faecal-oral transmission is a likely contamination route of STEC within the herd. In addition, the introduction of new animals to a herd may introduce STEC. The following are examples of measures that may be useful:

- segregate and limit faecal cross-contamination between newborn or young animals as well as with mature animals in the milking herd,
- keep young cattle in the same groups throughout rearing without introducing new animals.

15. Environmental transmission has also been demonstrated due to poor housing conditions or to the survival period of STEC (potentially more than a year) in effluent and the environment (soil, plants, crops, grain and water). Pastures can also maintain bacterial circulation by faeces deposited onto the ground and/or spreading of effluent. Apply good hygienic practices for manure and slurry management, with frequent removal from the milking herd environment and the maintenance of necessary intervals between spreading on pasture and the reintroduction of animals for grazing.

16. When appropriate, other control measures at primary production, such as diet, vaccination, administration of probiotics and additional good management practices (as described in the Raw Beef Annex) may be helpful in minimizing the shedding of STEC and, thus, contamination of raw milk, but more research on efficacy is needed.

17. Contaminated feed and water (surface water, roofing water, contaminated drinking water) can contribute to the introduction or circulation of STEC, following direct or indirect contamination. The presence of STEC in feed can be minimized by application of good manufacturing practices and appropriate manure and slurry management when the feed is produced on the farm (*Code of Practice on Good Animal Feeding* (CXC 54-2004)). Secure storage of feed is important to prevent STEC contamination from runoff water, pests and birds. In addition, it is important to limit water contamination for watering animals by adequate maintenance of water troughs.

5.2. Control measures for STEC during preparation of animals for milking, milking, and then transfer of milk to bulk containers/tanks.

18. The major route of raw milk contamination is from faecal sources (directly or indirectly). This in turn soils the teats, and consequently the milk can be subsequently contaminated during the milking process. Therefore, limiting faecal contamination during milking is of key importance to manage STEC on the farm. For this it is important to apply good hygiene practices during milking, to keep animals clean, and most importantly to prevent contamination with faeces.

Minimizing faecal contamination before and during milking:

- Manage a clean and hygienic environment for the milking animals to reduce faecal contamination. For example, the area where milking will be performed should be cleaned after each milking and allowed to dry when possible.
- Clean and disinfect all milking materials, utensils and equipment.
- Udders and teats should be properly cleaned before the milking process to minimize the risk of contamination of milk with STEC.
- In the case of manual milking, in addition to udder and teats, the operator's hands need to be properly cleaned.

19. STEC can also potentially persist on milking equipment and pipelines if these are not adequately cleaned and disinfected (Annex I Guidelines for the primary production of milk from CXC 57-2004). Cleaning and disinfecting are more challenging if equipment is not well designed for cleaning, and/or not well maintained. STEC can form biofilms in milking machines if they are improperly designed, poorly maintained and/or poorly cleaned. Studies have shown biofilm formation by O157:H7 STEC and non-O157 STEC with increased tolerance to sanitizers commonly used in the food processing environment, particularly if cleaning is not done effectively (resulting in biofilm formation in which the sanitizer cannot reach microorganisms) or the unintended application of a sanitizer at sub-lethal concentrations. All equipment that may come in contact with milking animal teats and milk as it is collected, such as milk collecting buckets, should be thoroughly cleaned and disinfected before every use. The hygienic quality of the water used for the last rinse is very important to prevent contamination of the milking machine (CXC 57-2004). In line with the *General Principles of Food Hygiene* (CXC 1-1969), only water fit for purpose (i.e., it does not cause contamination of the milk) should be used. If recycled water is used, it should be treated and maintained under conditions ensuring that its use does not impact the safety of the milk (CXC 57-2004).

6. CONTROLS DURING MILK COLLECTION, STORAGE AND TRANSPORTATION

20. If milk is processed immediately after milking, cooling is not necessary.

21. All equipment that may come in contact with milk, such as tubes and pipes used for transferring milk to larger containers, pumps, valves, storage containers and tanks, etc., should be thoroughly cleaned and disinfected before every use. Although not a standard practice, a full cleaning, once per 24 h, tanker cleaning approach, with the use of a between-load water rinse with or without a disinfecting treatment has been shown to reduce the presence of surface bacteria in the tanker, and thus may provide some risk reduction.

22. STEC can rapidly multiply in raw milk if the milk is at the temperature of STEC growth, so temperature control of the milk post-harvest is crucial, including during its storage at the farm and throughout the collection route to prevent microbial growth. Temperatures $\geq 6^{\circ}\text{C}$, extended storage of raw milk, and high initial bacterial counts in raw milk during collection, storage and transportation have been associated with increased counts of *E. coli* in raw milk. Milk temperature should be monitored during storage and checked before it is unloaded, when possible.

23. The stage of transport has not been identified as a step likely to contaminate the milk with STEC, if good hygiene practices are followed. Transport is also identified as a stage where growth of STEC may occur if the temperature of the milk is not properly maintained during transportation.

7. CONTROL DURING PROCESSING

24. The contamination of dairy products with STEC during processing in the manufacturing plants is rare if appropriate hygiene practices are followed. It is recommended that the products should be prepared and handled in accordance with the appropriate sections of the *General Principles of Food Hygiene* (CXC 1-1969), the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004) and other relevant Codex texts such as Codes of Hygienic Practice and Codes of Practice.

25. At the initial stages of cheese-making, the temperature (ranging from 27°C – 35°C) and a_w value of milk provide favorable conditions for the growth of STEC. During the first hours of cheese-making (transition from milk to curd), an increase in STEC level by 1-3 log can be observed in some cheese-making process. This increase in number is due to the multiplication of the cells in the liquid milk and then in the curd where cells are entrapped. However, “cooking” of cheese curd, as well as rapid acidification (when pH decreases to under 4.3), coupled to the increase of non-dissociated lactic acid, have been associated with STEC or *E. coli* log reductions of 1 to 4 log CFU/g. During the ripening step, the microbial stability of cheeses is determined by the combined application of different hurdle factors (pH, a_w , titratable acidity, sodium chloride, non-dissociated lactic acid, amount of starter cultures (such as lactic acid bacteria) still active in the cheese, brining of the cheese, as well as the temperature and length of time for ripening. These hurdles create an increasingly challenging environment for STEC during the manufacturing process and ripening. The food business operator (FBO) should analyze the risks associated with its manufacturing process with respect to the potential for growth or decline of STEC. Based on this assessment, the FBO should adapt the process and/or implement controls to reduce any identified risks for STEC contamination and growth.

26. “Cooking” of cheese curd, rapid acidification or long ripening may not be compatible with some traditional production practices, as they may impact the sensory characteristics of the cheese. In such cases other control measures should be identified and applied. For example, testing raw milk for the presence of STEC can be established, as well as an audit program of milk suppliers to assess their hygienic practices.

27. Nevertheless, these procedures have the potential to reduce the number of STEC, but they cannot ensure the safety of the product if the raw milk is contaminated with STEC. Consequently, the microbiological quality of raw milk used in cheese making is crucial for reduction of the risk associated with the end products.

8. PRODUCT INFORMATION FOR CONSUMERS

28. In line with the *Code of Hygienic Practice for Milk and Milk Products* (CXC 57-2004, section 9.1), raw milk products should be labelled to indicate they are made from raw milk according to national requirements in the country of retail sale.

9. VALIDATION, MONITORING AND VERIFICATION OF CONTROL MEASURES

9.1 *E. coli* enumeration and STEC testing

29. Although STEC can be isolated from raw milk and raw milk cheeses, STEC testing is uncommon and most sampling and testing protocols target indicator microorganisms such as *E. coli*, whose level can be used as an indicator of raw milk sanitary quality prior to raw milk cheeses production. Microbiological criteria (refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Relating to Food* (CXG 21-1997)) based on process and hygiene indicator microorganisms (e.g., *E. coli* / Enterobacteriaceae) may also prove a useful tool for validation, monitoring and verification of control measures.

30. Even if they are useful hygienic markers of the quality of raw milk, the presence or concentration of generic *E. coli* or other indicator microorganisms in raw milk does not indicate the presence of STEC. More specific analyses are needed to detect and confirm by strain isolation the presence of STEC. Periodic testing for STEC strains considered to be a country's highest priority (e.g., those strains with virulence factors capable of causing severe illness or considered to cause significant illness in that country) may also be conducted for verification of hygienic practices.

31. Testing raw milk for the presence of STEC strains considered to be a country's highest priority can be established, but testing may not be effective on its own: because of the low prevalence of STEC, samples tested may not contain STEC despite their presence in the food. Thus, such testing should be used in combination with other control measures, including an audit programme of milk suppliers to assess hygienic practices on the farm.

9.2. Validation and monitoring of control measures

32. Control measures should be validated before being implemented. To limit the cost of this important step, it can be shared by several FBOs and a professional organization which may gather, analyze and interpret data in order to establish alternative or improved measures, for example by writing GHP guidelines adapted to the local context or to the traditional steps of raw milk harvesting and processing.

33. The description of control measures may also include the procedures for monitoring their implementation to ensure the control measures are carried out as intended.

9.3. Verification of control measures

34. **At the dairy farm:** Testing periodically for microorganisms that are indicators of faecal contamination or hygiene in milk can be implemented. For example, routine analysis of milk at the point of production for microbial quality indicator microorganisms (*E. coli*, coliform levels or total aerobic plate counts) can provide information on the hygiene of the operation. Nevertheless, low levels of indicator microorganisms do not confirm the absence of STEC nor other pathogens.

35. Enhanced monitoring should be implemented when STEC strains have been detected in milk or in cheeses and production and sale of the products should be ceased until the contamination issue has been resolved. In such situations input from technical experts or professional association guidance, as well as guidance from competent authorities, can help to identify the risk factors for milk contamination. Finally, a criterion should be defined for when to return to routine monitoring. This criterion should be based on experience and statistical evaluation of the history of microbiological analyses results.

36. General hygiene audits can be useful to check periodically that the GHPs are effectively implemented at each farm where the milk is collected. They might be conducted by the dairy establishment or by a local professional association.

37. **Milk collection to the dairy establishment:** Routine surveillance of the quality of the raw milk received by the dairy establishment (indicator microorganisms or/and STEC) conducted by the dairy establishment can be based on samples collected periodically or even for each load. Sampling milk filters may be a more suitable monitoring point for STEC than sampling raw milk from the bulk tank, considering dilution due to pooling and

sporadic contamination issues. Milk filter sample analysis can also be useful in investigating the source of contaminated cheese.

38. Enhanced surveillance of all the suppliers can be set up when STEC strains have been detected in mixed milk unloaded at the processing plant. In such a situation, another measure could be to increase the frequency of sampling and STEC analysis in order to assess the milk origin of the strain, the magnitude of contamination and the persistence of the strains in the processing plant. Then, criteria to return to routine monitoring should be defined.

39. **During processing:** A milk quality check based on STEC detection is an option that some FBOs may consider for raw milk (STEC negative milks). This approach can nevertheless be difficult because of the complexity, the time taken and the cost to analyze for STECs in milk. Alternatively, milk quality checks can be performed based on *E. coli*, to verify the application of good hygienic practices.

40. Sampling and testing of raw milk cheeses are an important part of verification plans, to confirm that practices and procedures described in the food safety programme are successful. Accurate safety and quality test results are crucial and depend on appropriate sampling and sample handling, the type of representative samples, and proper methods. For routine surveillance, FBOs should consider analyzing cheese during the early stages of manufacturing, when the peak of STEC growth is likely to take place. Testing at this time would have a greater sensitivity than end product testing and would save producers the expense of aging and storing contaminated products. Analysis could also be done during ripening and / or before placing the cheese on the market.

41. When STEC are accidentally present in raw milk, it has been found at very low levels in cheeses. This contamination is characterized by heterogeneous distribution, making STEC difficult to detect. Sampling plans should therefore be designed according to the *General Guidelines on Sampling* (CXG 50-2004). In addition, sampling plans should be adapted over the entire production chain (number of samples, nature of the samples (for example: milk, cheese at the start of coagulation, during ripening, etc.), quantity analyzed, frequency of analysis, etc.).

42. The FBO or industry association generally defines its sampling plan in line with an acceptable sanitary quality level.

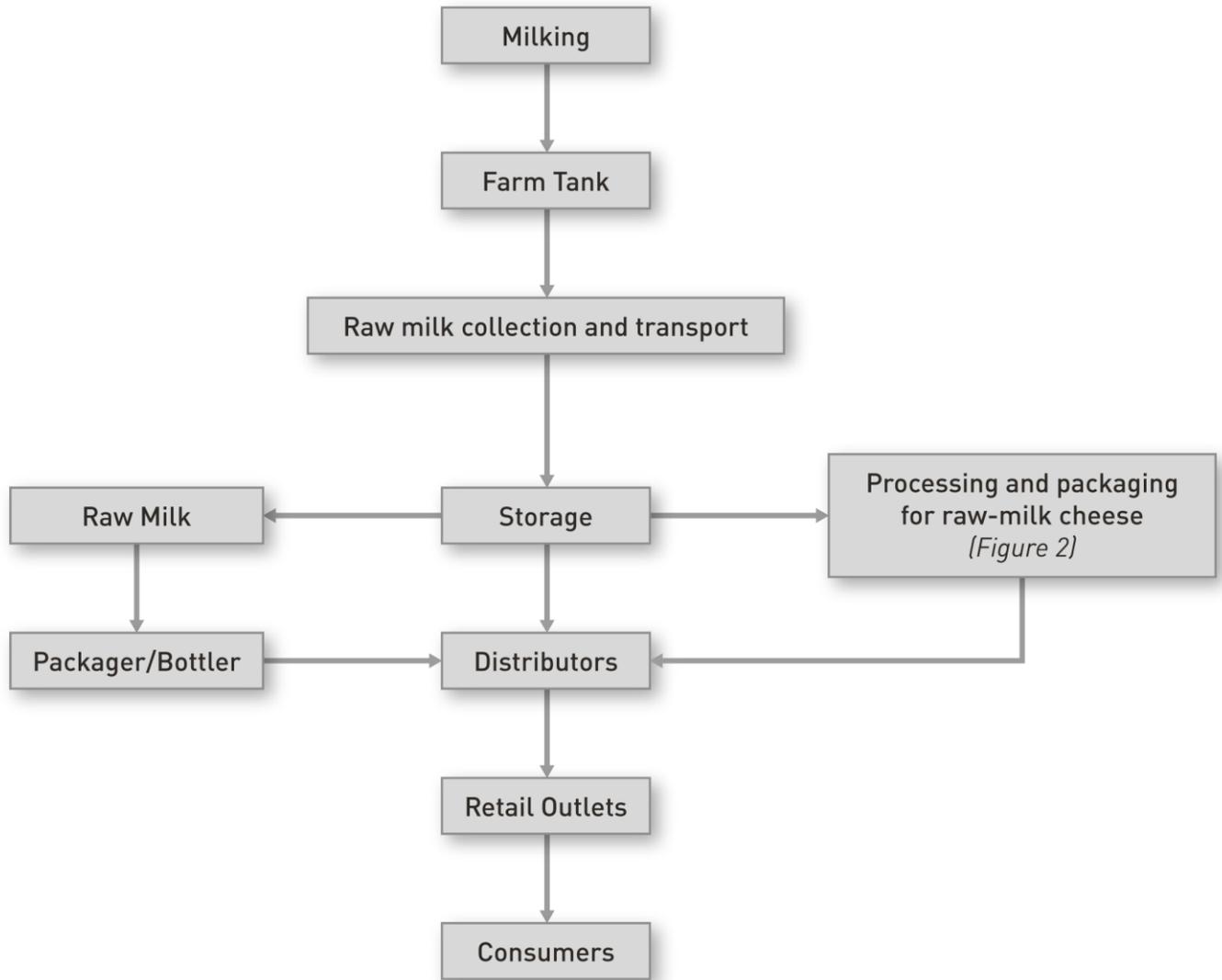
43. Enhanced surveillance can be put in place when STEC are detected in curds or in cheeses or in the case of a public health risk. For example, other batches of cheeses can be screened in greater detail for STEC to assess the magnitude of contamination. In addition, it is important to identify the remaining contaminated milk, if any, and stop using it.

44. **Quantitative risk assessment:** Several sampling plans may be applied at different steps (milk harvested at the farm, milk delivered at the dairy establishment, curds, final products). Their combination in a quantitative risk assessment (QRA) model can help assess the efficacy of this sampling plan, using simulation, in terms of risk reduction of illness and percentage of batches rejected. Specific QRA models for STEC in several raw milk cheeses matrices have been developed. QRA models can also be built based on databases obtained when combining results of microbiological analyses performed regularly on the milk at different levels (farm and tank) and on cheeses (during the process and on the final product), values on technological process parameters and physico-chemical values (e.g., pH, a_w) on the capacity for growth or survival of the microorganisms considered.

45. QRA models can help compare sampling plans to determine which one provides better protection.

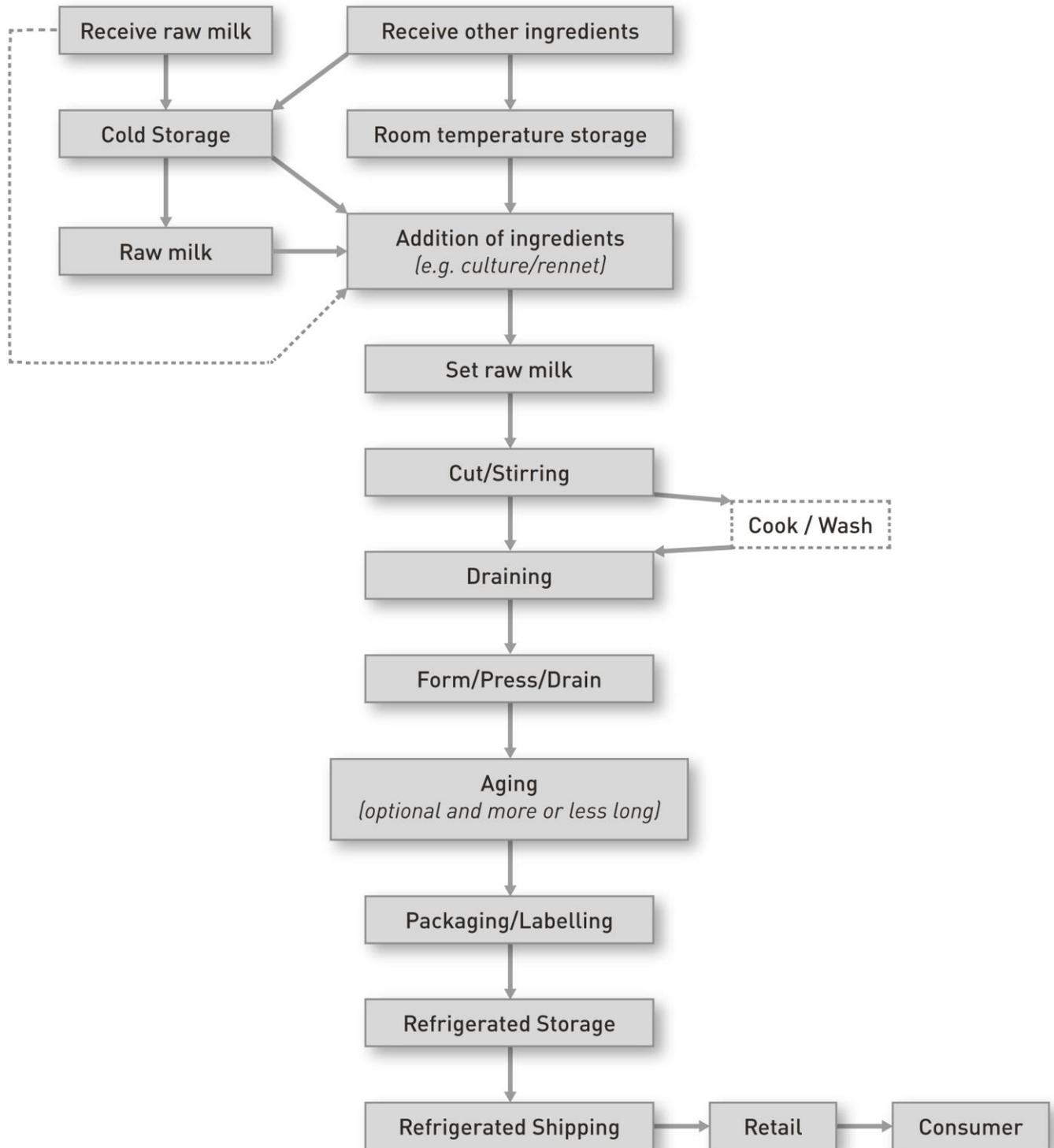
46. **Application of prerequisite programmes, including good hygiene practices, and HACCP principles:** Given the low frequency and low level of contamination by STEC strains and the limits of the sampling plans, it is the combination of control measures (including GHPs and HACCP, when applicable) throughout the dairy chain that will reduce the risk of STEC contamination of the products put on the market.

Figure 1. Process Flow Diagram for Raw Milk Production, Distribution and Sale



The diagram illustrates a generalized process flow raw milk for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram.

Figure 2: Making Cheese from Raw Milk



The diagram illustrates a generalized process flow raw milk for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram.

SPROUTS

1. INTRODUCTION

1. Sprouts are commonly consumed raw and most often there is not a kill step applied that would eliminate microbial pathogens, prior to consumption. Consequently, it is necessary to ensure safe production of sprouts by preventing or minimizing contamination of incoming seeds, in the production environment and in the finished products. While no single step will reliably eliminate all pathogenic microorganisms that may survive on sprouts, using a multi-hurdle approach implementing a series of preventive and risk-reduction steps can greatly reduce the food safety risks that may be associated with sprouts.
2. Sprouts have different food safety concerns from other fresh fruits and vegetables because the conditions for seeds to sprout (time, temperature, water activity, pH and available nutrients) also support the growth of foodborne bacterial pathogens if present.
3. Contaminated seeds have historically been identified as the likely source of most sprout-related outbreaks, particularly those attributed to Shiga toxin-producing *E. coli* (STEC) contamination and continues to be the most common source of sprout contamination (NACMCF, 1999; EFSA, 2011; Ferguson et. al., 2005, FAO/WHO, 2022). Bacterial pathogens that may be present at low levels on seeds can multiply to very high levels during the sprouting process. Sprout contamination could also be caused by poor hygienic practices and contamination in production environments (FAO/WHO 2022).
4. Figure 1 provides a flow diagram illustrating a generalized process flow to produce sprouts. This flow diagram is for illustrative purposes only. Steps may not occur in all operations in grey line and may not occur in the order presented in the flow diagram. Sprouts are grown in production environments that vary based on size and resources of the operation, seeds type, available equipment, etc.
5. During seeds production, conditioning and storage, the application of Good Agricultural Practices (GAPs) and Good Hygienic Practices (GHPs) should aim to prevent the contamination of seeds by microbial pathogens such as STEC. During sprout production, the microbiological decontamination of seeds step is aimed at reducing potential contaminants and the GHPs at preventing the introduction of microbial pathogens and minimizing their potential growth. The degree of control in these two areas has a significant impact on the safety of sprouts.

2. OBJECTIVE

6. The objective of this Annex is to provide guidance to reduce the risk of foodborne illness from STEC associated with sprouts during production, harvesting, packing, processing, storage, and distribution.

3. SCOPE, USE, AND DEFINITIONS

3.1 Scope

7. This Annex covers specific guidance for the control of STEC related to sprouts that are intended for human consumption without cooking.
8. Home-sprouting, and shoots, cress, and microgreens²⁹ where the seed is not kept in the final product are outside the scope of this document.

3.2 Use

9. This Annex should be used in conjunction with the *General Principles of Food Hygiene* (CXC 1-1969) and the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003), including Annex II for Sprout Production.

3.3 Definitions

²⁹ Shoots are grown hydroponically, and true leaves are developed. The shoots and the leaves are cut during harvest and the final product does not include the seed and roots. Cress is grown with substrate and true leaves are developed; as with shoots grown hydroponically, the cut shoots and leaves do not include the seed and roots. For microgreens, plants reach a later stage of growth than sprouts, typically associated with the emergence of “true” leaves. They can be grown in soil or substrate and are harvested above the soil or substrate line; they include both shoots and cress (FAO/WHO, 2022).

Sprouts - Sprouted seeds or beans harvested when the cotyledons (or seed leaves) are still un- or underdeveloped and true leaves have not begun to emerge. They can be grown in water, soil or substrate and can be harvested with or without the root (cut sprouts)³⁰

Seeds for sprouting – Seeds or beans used to produce sprouts for human consumption.³¹

4. PRIMARY PRODUCTION OF SEEDS / BEANS FOR SPROUT PRODUCTION

4.1. Control measures for seed production and handling

10. Interventions aimed at reducing the risk from seed-borne contamination should focus on controlling contamination of seeds from animal and human activities and ensuring proper use and application of manure, biosolids, other natural fertilizers, and agricultural water.

4.1.1. Animal and human activities

11. Grazing of domestic animals should not occur in fields while crops are actively being grown for sprout seed production. History of the growing area regarding previous uses for grazing domestic animals should also be considered, as STEC may survive for several weeks in bovine feces.

12. In addition, nearby fields with livestock can increase the risk of STEC contamination. Livestock should be located as far as feasibly possible from fields growing sprouted seed, because the risk decreases as the distance of livestock increases (Berry et al., 2015, 2019).

13. Growers should assess during the growing season the areas used for growing seed for sprouting for evidence of potential contamination of seed from domesticated or wild animals (e.g., observation of animals, animal excreta, crop destruction).

14. When evidence of potential contamination is found (e.g., the plant or seed is visibly contaminated with animal excreta), growers should evaluate whether the seed should not be harvested due to the potential for contamination with STEC. Growers should then take measures to identify contaminated seed and/or the contaminated area (e.g., mark the affected area) so that it will not subsequently be harvested even if weather events, or other occurrences make the excreta not visible at that time.

15. Wild animals should be excluded from the production area to the extent possible.

16. The presence of nearby animal production facilities (e.g., animal feed operations, poultry farms, dairy farms) or other related factors such as slope of land, lack of runoff controls, and manure spreading that could lead to contamination of the seed or irrigation water with untreated manure should be assessed and appropriate actions taken to prevent contamination of growing areas and seed with STEC.

4.1.2 Water for seed production

17. Water for irrigation and other applications should be fit for purpose and used in a manner to avoid the introduction of pathogens onto seeds.

18. Growers should evaluate the sources of water used on the farm for the likelihood of contamination with STEC (e.g., from livestock, wildlife, sewage treatment, human habitation). The following actions may prevent contamination of water supplies with STEC:

- installation of fencing around surface water supplies to prevent large animal contact,
- proper maintenance of wells,
- water filtering or chemical water treatment,
- prevention of stirring of the sediment when drawing water,
- construction of settling or holding ponds or water treatment facilities.

19. The effectiveness of these actions should be verified by periodic water testing. Where necessary, growers should test the water they use for appropriate indicator microorganisms and, where necessary, STEC, according to the risk associated with the production. The frequency of testing will depend on the water source (e.g., lower for adequately maintained deep wells, higher for surface waters), the risks of environmental contamination,

³⁰ FAO/WHO. 2022. Microbiological Risk Assessment Series 43: *Prevention and control of microbiological hazards in fresh fruits and vegetables – sprouts*.

³¹ References to “seeds” in this document include other things that are sprouted to produce sprouts for human consumption, such as beans.

including intermittent or temporary contamination (e.g., heavy rain, flooding), or the implementation of a new water treatment process by growers.

20. Where possible, growers should have a contingency plan in place that identifies an alternative source of fit-for-purpose water if the primary water source is found to have unacceptable levels of indicator microorganisms or is contaminated with STEC.

4.1.3 Manure, biosolids and other natural fertilizers

21. Growers who use biological soil amendments of animal origin (e.g., manure) on fields producing seeds for sprouting should only use them in such a way that they do not contaminate the seeds for sprouting. Manure, biosolids, and other natural fertilizers are potential sources of bacterial pathogens. Only composted manure/biosolids treated to reduce or eliminate STEC should be used during seed production to reduce the risk of seed contamination.

22. Extending time intervals between application of treated manure/compost/biosolids and harvest of seeds may also decrease the risk of seed contamination.

4.1.4 Personnel health, hygiene, and sanitary facilities

23. Worker hygiene and health requirements should be followed to ensure that personnel who have direct contact with seeds for sprouting prior to, during or after harvesting will not contaminate them with STEC.

24. Adequate access to, and use of, hygienic and sanitary facilities, including adequate means for hygienically washing and drying hands, are critical to minimize the potential for workers to contaminate seeds for sprouting.

25. People known or suspected to be suffering from diarrheal illness should not be allowed to enter any area handling seeds destined for sprouting, including the growing and harvest area.

26. Please also refer to the General Principles of Food Hygiene CXC 1-1969 section 3.2.3 and section 6 for more recommendations that may apply.

4.1.5 Equipment associated with growing and harvesting of seeds for sprouts

27. Equipment should be designed and maintained to minimize soil intake and seed damage, and prevent introduction of pathogens such as STEC onto seeds

28. Growers should avoid moving harvesting equipment across fields where manure or compost has been applied.

29. Harvesting equipment should be cleaned and disinfected if, for example, the equipment runs over an area with animal intrusion and faecal deposits, to avoid contamination of seeds destined for sprouting. Equipment should always be cleaned and disinfected prior to harvesting.

4.1.6 Handling, storage, and transport of seeds for sprouts

30. Temperature and humidity should be controlled, and good hygiene practices (GHPs) implemented to avoid possible contamination of seeds during storage and transportation.

31. Equipment used to transport the seeds should be clean and, where necessary, disinfected prior use.

32. Packaging of seeds is recommended to minimize the potential for contamination. Growers should pack and hold seeds under sanitary conditions and pest controls should be implemented in storage facilities.

33. Seeds should be stored in closed or covered containers, in a clean, dry area dedicated only to seed storage.

34. Containers should not be stored on the floor and not be placed against walls to reduce the possibility of contamination with STEC by rodents or other pests and to facilitate regular monitoring for pest problems

35. Containers stored in the outdoors should be cleaned and, as appropriate, disinfected before being used to transport seeds for sprouting and be positioned off the floor.

36. Use solid bags to hold seeds for sprouting - open weave bags should not be used.

37. Avoid using contaminated or recycled bags.

38. Mark each container to identify source and lot. For any seed that has been treated, clearly state this on the label.

5. SPROUTS PRODUCTION

39. HACCP principles should be applied to sprout production, with all the steps well documented and potential critical control points (e.g., decontamination of the seeds) identified and controlled. If a problem is identified (e.g., STEC contamination of sprouts), corrective actions should be taken and a critical review of all the steps should be performed to determine whether changes are needed. Not mixing seeds and sprouts from different batches can facilitate the identification of batches with problems and tracing seeds back to the supplier. Water used throughout sprouts production should be fit for purpose.

5.1 Reception of sprout seeds / beans

40. Where feasible, seeds should be obtained from suppliers, (producers or distributors) that follow GAPs and GHPs during production, storage, distribution, and commercialization of the seeds. When possible, microbiological testing/certificates of analysis or a letter of guarantee should be requested from the supplier.

41. When seeds arrive at a sprout operation, they should be inspected for physical damage and signs of contamination (e.g., rodent/bird droppings, dirt, and other visible contamination).

5.2 Storage of sprout seeds / beans

42. Once received, seeds should be stored and handled in a manner that will avoid damage, prevent growth of microorganisms such as STEC, and protected from pests and other sources of STEC contamination.

5.3 Initial Rinse

43. The seeds should be rinsed thoroughly before any antimicrobial treatment to remove dirt and increase the efficiency of the antimicrobial treatment.

44. Rinse and agitate seeds in large volumes of potable water. Repeat the process with potable water until most of the dirt is removed and rinse water remains clear.

45. Carry out the rinsing process in such a way to maximize surface contact of seeds with water (e.g., use large buckets of water and sieves).

5.4 Sprout seed treatment and pre-germination soak

46. Treatment of seeds to reduce the presence of pathogens such as STEC is a potential critical control point. However, seed treatment can be challenging due to the low water activity of the seeds, and the need to preserve the viability of the seeds, including their ability to germinate. Therefore, because treating seeds used for sprouting reduces contamination (Montville et al., 2005; Fett, 2002) but does not guarantee pathogen-free sprouts, efforts should be made to avoid contamination.

47. Known seed treatment methods include those that work by chemical means (liquid or gas), physical means, or a combination of these. The use of certain seed treatments may be subject to approval by competent authorities.

48. The following chemicals, when used at appropriate concentrations, may be able to achieve at least a 3-log reduction of pathogens: calcium hydroxide (Holliday et al., 2001), calcium hypochlorite (Ding et al., 2013), sodium hypochlorite, (Ding et al., 2013) caprylic acid (Chang et al., 2010), gaseous acetic acid (Nei et al., 2011; Nei et al., 2014), hydrogen peroxide (Holliday et al., 2001), lactic acid (Sikin et al., 2013), monocaprylin (Chang et al., 2010), oxalic acid (Sikin et al., 2013), and phytic acid (Sikin et al., 2013) When using chemical treatments, accurately measure and record the duration of treatment and the concentration of the chemical used.

49. Physical treatments have been reported to achieve a 5-log or greater reduction in pathogens, including *E. coli* O157:H7, on seeds (Bari et al, 2010, Ding et al., 2013, Neetoo et al., 2013). Physical treatments, such as heat (dry heat or hot water), high pressure, and irradiation are reported to have better penetration characteristics for reaching bacteria on microscopically rough surfaces as well as the interior of the seed as compared to chemical treatments (Ding et al., 2013). Physical and combination treatments have been reported to be the most effective for removing pathogens from seeds for sprouting. Combination methods are recommended, where feasible, since applying two or more methods sequentially or simultaneously may be more effective than using a single treatment alone.

50. Where feasible, sprout growers should treat the seeds used for sprouting with a method validated to reduce microorganisms of public health significance such as STEC.

51. All steps involved in antimicrobial treatment for seeds should be carried out in an area separated from the germination and packaging areas.

52. After treatment, seeds are generally soaked for up to 12 h in water to soften hulls and improve germination.

5.5. Rinse after seed treatment

53. Seeds may need to be rinsed after a seed treatment (e.g., seeds treated with chemicals). Time of rinse should be adequate to limit potential microbial growth.

5.6. Germination and Growth of sprouts

54. Sprouts are grown hydroponically or in soil. Practices employed for germination, growth, harvest, and post-harvest washing vary depending on the operation and the type of sprout grown. Growing units include rotating drums, bins, beds, trays, and buckets.

55. Seeds for soil-grown sprouts are generally rinsed and soaked to allow for initial germination before sowing in soil in plastic trays. Water is sprayed over the trays daily. Sprouts such as alfalfa, broccoli, clover, and radish are grown hydroponically in rotating drums with frequent water sprays. If present at the growing stage, microbial pathogens such as STEC can multiply, significantly increasing the risk for illness.

56. In addition to the seed treatment methods described above, research has also indicated a novel growing method, e.g., growing sprouts at 4.4 °C following a 2,000 ppm sodium hypochlorite seed treatment, can result in a decrease in *E. coli* O157:H7, and a significant increase in product shelf life (Loneragan et al., 2018).

5.7 Harvesting

57. Sprouts are harvested manually by removing them from growing units. Sprouts may be washed to remove hulls and/or to help lower the temperature of the sprouts and then spin-dried. Soil-grown sprouts are harvested by cutting them from the trays, prior to washing and packaging, or the sprout trays are sent to retailers and cut at the point of sale. GHPs should be applied to prevent these operations from being source of contamination (e.g., if some of the sprouts are contaminated with STEC from the environment or from handlers).

5.8 Cold sprout storage

58. Sprouts should be maintained at appropriate temperatures³² after cooling to minimize growth of any STEC that may be present. The temperature of the cold storage should be controlled, monitored, and recorded.

5.9 Personal and environmental Hygiene at sprout production

59. Proper storage, handling and disposal of waste, sanitation of equipment and tools, and effective pest control will minimize the risk linked to sprout contamination with pathogens such as STEC.

60. Proper facility design (e.g., differentiation between areas, zones) and operation and employee flow to avoid raw material in contact with the final product will reduce the risk of cross-contamination.

5.10 Documentation and records

61. Documentation of key information for incoming seeds (e.g., supplier details, date of receipt, quantity etc.) should be maintained.

62. It is recommended that harvesting, production, and distribution records should be retained long enough to facilitate STEC illness investigation and recalls if needed. This period may significantly exceed the shelf-life of sprouts.

63. It may be appropriate to retain microbiological test results for a longer period since this data may be used to look for trends (through trend analyses) in indicator levels. Increases over time can suggest that there is an emerging issue (or issues) in the production process which may require remediation.

64. Refer to section 5.7 of the *Code of Hygienic Practice for Fresh Fruits and Vegetables* (CXC 53-2003) for the types of records that should be maintained by growers, harvesters and packers that may be important when investigating foodborne illness outbreaks due to STEC.

6. MICROBIOLOGICAL CRITERIA AND OTHER SPECIFICATIONS FOR LABORATORY TESTING

65. It is recommended that sprouts or spent irrigation water, and possibly seeds, be tested for the presence of pathogens such as STEC. Refer to the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CXG 21-1997).

6.1 Testing of seed lots before entering production

³² A temperature of 7°C or below will prevent growth of STEC.

66. Testing lots of seed for sprouting for pathogens such as STEC can help identify contaminated lots; thus, some seed producers may opt to test their seed for pathogens before distribution. However, the likelihood of detecting the presence of pathogens such as STEC in seeds is low, due to the heterogeneous distribution and low numbers of STEC contaminating the seeds. Thus, a negative test does not assure the absence of STEC on the seeds. Testing for indicator microorganisms may be used as an indicator of the general level hygiene of seeds prior to production.

6.2 Testing of sprouts and/or spent irrigation water

67. Microbial testing of spent sprout irrigation water (or in-process sprouts) is an important part of a multi-hurdle approach to ensure contaminated sprouts do not enter the marketplace. Testing spent sprout irrigation water (or in-process sprouts) for STEC from each production batch of sprouts may be a much more reliable indicator than testing seed to determine whether the sprouts, and the seeds used to produce the batch, are contaminated with STEC.

68. Samples of spent irrigation water can be collected as early as 48 hours after the start of sprouting. If the seeds are pre-soaked (e.g., soaked in water for a short time and then transferred to growing units for sprouting), include the pre-soak time. Early results will allow sprout growers to take corrective actions sooner, thus minimizing the potential for one lot of sprouts to contaminate other lots.

69. If testing spent sprout irrigation water is not practicable (for example, soil-grown sprouts harvested with roots or for hydroponically grown sprouts that use very little water), each production batch of sprouts could be tested at the in-process stage (i.e., while sprouts are still growing).

70. The highly perishable nature of sprouted seeds generally makes routine microbiological testing of end-product impractical. Testing of seed lots spent sprout irrigation water, or in-process sprouts is more practical. However, periodic testing of end product for *E. coli* may have benefit for evaluating the overall effectiveness of hygiene practices and post sprouting treatments (e.g., final rinse).

7. DISTRIBUTION AND POINT-OF-SALE

71. STEC growth and contamination can occur during transport, distribution and at point-of-sale due to improper handling and poor personal hygiene, and contamination through comingling with raw commodities and animals/animal products, and exposure to unsanitary surfaces and water. Control measures should be applied during distribution and at point of sale to prevent contamination with STEC.

7.1 Transportation

72. Transportation should be done in clean, enclosed, and refrigerated transport vehicles and temperature should be monitored.

8. PRODUCT INFORMATION AND CONSUMER AWARENESS

73. Producers should provide relevant information to the consumer to assure the safety of sprouted seeds during storage, handling and preparation of the product, to include: (1) recommended temperature of storage; (2) use-by date; (3) cooking instructions, which should be included on the label if the product is intended to be consumed as non-RTE.

74. Consumers should hold sprouts at temperatures they will minimize the growth of pathogens such as STEC and adhere to the use-by date provided.

9. TRAINING

75. All personnel involved in the production and handling of seeds for sprouting or sprouts across the supply chain should receive training on the principles of food hygiene and food safety as well as personal health and hygiene requirements.

76. Seed producers, handlers, distributors, and processors should be aware of GAPs, GHPs and their role and responsibility in protecting seeds intended for sprouting from STEC contamination.

77. Interventions designed to reduce microbiological hazards in sprouts can be highly technical and difficult to implement. Specific training related to seed sourcing and storage, seed treatment, cleaning and sanitizing, sampling and microbiological testing, and record keeping should be done to ensure successful implementation.

10. RETAIL AND FOODSERVICE

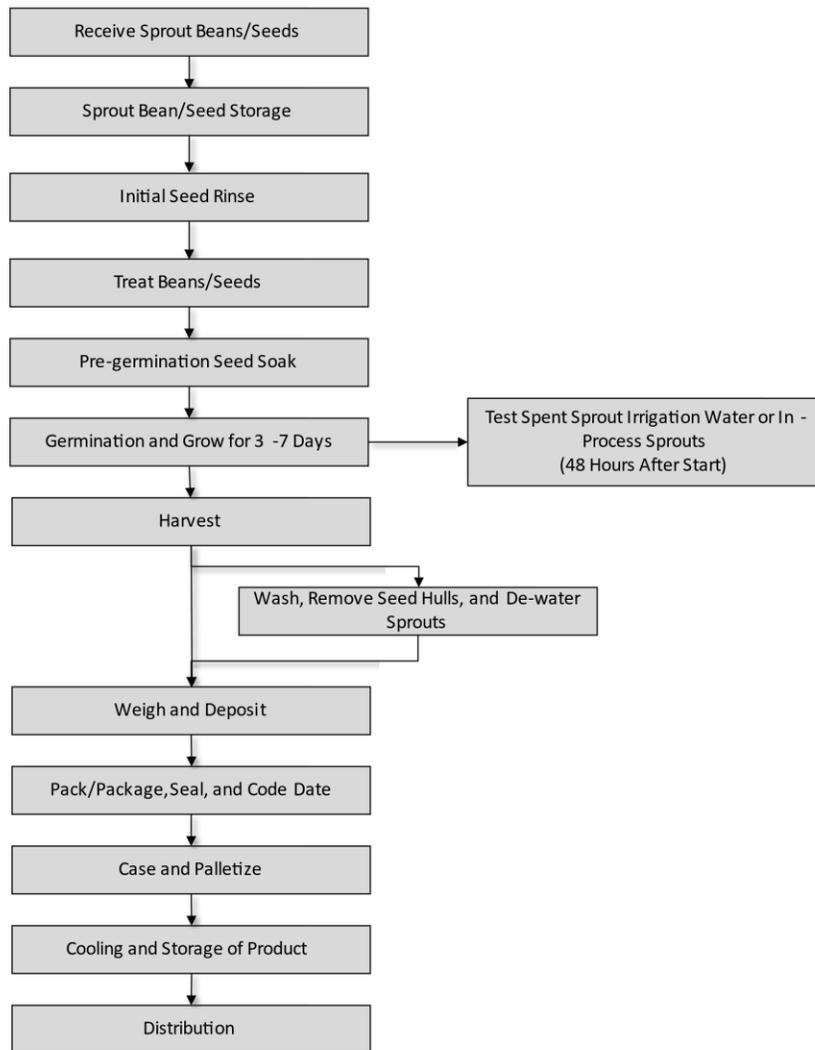
78. Sprouts for retail sale should be held at an appropriate temperature to minimize growth of STEC. Temperatures should be monitored.

79. Food business operators serving sprouts for consumption without cooking to consumers should take appropriate measures to:

- prevent cross-contamination,
- maintain sprouts at an appropriate storage temperature to minimize growth of STEC that may be present, and
- ensure proper cleaning of tools and surfaces that may come in contact with these products.

80. For in-restaurant sprouting, interventions recommended for sprout operations to minimize the potential for STEC should be considered, including seed sourcing programs, seed treatment (if appropriate), sampling and testing of spent sprout irrigation water (samples to be tested by contract laboratories), as well as cleaning and sanitizing food contact surfaces.

Figure1: Sprouts Flow Diagram³³



³³ The diagram illustrates a generalised process flow to produce sprouts for illustrative purposes only. Steps may not occur in all operations and may not occur in the order presented in the flow diagram and the germination time may be different.

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Appendix II

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