



**Australian Government**  
**Department of Agriculture,  
Fisheries and Forestry**

# **Microbiological Manual for Sampling and Testing of Export Meat and Meat Products**

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**Department of Agriculture, Fisheries and Forestry**

## Disclaimer

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## 1. INTRODUCTION

Export legislation<sup>1</sup> requires all export registered establishments producing meat and meat products to comply with the relevant Australian Standard and to satisfy importing country requirements. The *Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption* (AS 4696) requires that the establishments must participate in surveillance, sampling, monitoring and testing programs to ensure that meat and meat products are safe for human consumption. The Department of Agriculture, Fisheries and Forestry (DAFF) verifies compliance and certifies that product from export registered slaughter, boning and further processing establishments was produced in accordance with these requirements.

The Australian Export Meat Inspection System is designed to provide objective measurements to substantiate claims of food safety and wholesomeness and to provide meaningful data for monitoring process controls. The Australian system also requires that establishments monitor their performance against key performance indicators (KPIs), identify non-compliance in process control and implement corrective and or preventive action for non-compliance.

The Meat Standards Committee developed guidelines for microbiological testing for the purpose of demonstrating process control pursuant to the Australian Standards. They required:

- Testing of product and working surfaces
- Keeping records of test results
- Monitoring results by graphing them over time
- Taking action if an adverse trend develops.

This document does not provide guidance for the microbiological testing of work surfaces or personal equipment. Registered establishments should follow the requirements in the Meat Standards Committee document [\*“Microbiological Testing for Process Monitoring in the Meat Industry” \(2002\)\*](#) when developing their work instructions for sampling of contact surfaces. Laboratories undertaking surface testing should comply with the [\*“General Requirements for On-Plant Laboratories”\*](#).

This document summarises the requirements for the export certification testing of meat and meat products at export registered establishments.

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<sup>1</sup> *Export Control (Meat and Meat Products) Rules 2021*

## 2. PURPOSE

To provide advice on the requirements of the microbiological sampling and testing programs in which export registered meat establishments (including wild game meat and meat processing establishments) must participate to comply with the requirements of the Australian Export Meat Inspection System. This manual includes both Australian and importing country requirements including the verification requirements for the export of Australian meat and meat products.

## 3. SCOPE

This document applies to:

- Export registered establishments that produce meat and meat products from animals including:
  - o Export registered slaughterhouses
  - o Export registered independent boning rooms
  - o Export registered game processing establishments
  - o Export registered further processing facilities
- Approved laboratories that conduct microbiological and parasitological testing for export meat establishments (on-plant and independent laboratories)
- Department officers conducting or verifying microbiological testing at export registered establishments or laboratories.

Microbiological testing programs covered in this manual are those programs approved by the department to demonstrate compliance with the relevant Australian standards and to meet certification requirements for export meat and meat products.

## 4. DEFINITIONS AND ABBREVIATIONS

Definitions in this manual are as per the relevant Australian Standard, except where specified otherwise.

APC	Aerobic Plate Counts (sometimes referred to as Total Viable Count - TVC or Total Plate Count - TPC)
CFU	Colony Forming Unit (a colony on a plating media at the time of counting)
ELMER	Electronic Legislation, Manuals and Essential References
ESAM	Escherichia coli & Salmonella Monitoring Program
EU	European Union
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service (USA)

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HACCP	Hazard Analysis and Critical Control Point
KPI	Key Performance Indicator
MEDC	Meat Export Data Collection System
NATA	National Association of Testing Authorities (Australia)
NCMMP	National Carcase Microbiology Monitoring Program
PHI	Product Hygiene Indicator (program)
RTE	Ready-to-eat
SRA	State Regulatory Authority

## 5. LABORATORY REQUIREMENTS

### 5.1. Approval

All laboratories undertaking testing under the scope of this manual must be approved by the department. Laboratories must apply in writing to the department for approval.

To be approved, laboratories must be accredited under ISO/IEC 17025 *General requirements for the competence of testing and calibration laboratories* by the National Association of Testing Authorities (NATA), or where this is not required, meet the relevant requirements and responsibilities as detailed in the “[General Requirements for On-Plant Laboratories](#)” and the “[Approved Laboratory Program](#)”.

A list of [approved laboratories](#) is available on the department website: [ELMER3](#).

### 5.2. Test Methodology

Department approved methods must be used for testing of samples covered under the scope of this manual. An updated [list of methods](#) and [relevant documents](#) are available on the department website.

Laboratories, establishment management or method developers may seek approval for a method not currently listed by writing request to the department. Methods will only be approved if they are:

- Appropriately validated (validation data to be provided to the department in English)
- Appropriate for the product and organism being considered
- Considered by the department to be acceptable to the competent authority of the intended importing country
- Intended to be used in a department approved laboratory.

Laboratories should also conduct a verification study for approved methods prior to their first use to verify that laboratory operators using their equipment in their working environment achieve the same results as defined in the validation data for that method. Verification studies should be consistent with NATA guidance on verification of test methods.

Laboratories accredited by NATA should seek approval to use a new method firstly from NATA prior to seeking approval by the department..

Methods submitted to the department for approval may need to be reviewed by importing country authorities before they can be listed for use in microbiological testing undertaken as part of the department certification program.

Variation or modification of methods is not permitted unless approved and specified in writing by the department.

### **5.3. Assuring the quality of test results**

Laboratories must have appropriate documented procedures for monitoring the validity of test results including media quality control and verification of test results as per relevant standards and guidelines. See "[General Requirements for On-Plant Laboratories](#)" and the "[Approved Laboratory Program](#)".

Laboratories must be furnished with all equipment necessary to ensure the validity of tests. Equipment must be properly maintained and calibrated as per relevant standards and NATA guidelines where applicable.

Control cultures must be used in daily test and media quality control. Laboratories must have documented procedures for handling, maintaining, preparing and use of control cultures.

### **5.4. Laboratory Competency**

The department requires that all approved laboratories participate in a recognised Proficiency Testing (PT) program (where available) at least bi-annually for each test covered by their scope of approval.

Laboratories must agree to the release of PT results directly to the department from the PT provider.

The results of the PT program will be used by the department to assess laboratory competence, highlight possible training needs and as part of ongoing approval decisions.

## 5.5. Reporting Results

Laboratory results for samples analysed under the programs detailed in this manual must be reported independently to the department and before or at the same time that they are reported to the establishment or the establishment manager (person who manages and controls operation on site).

The establishment manager is to provide laboratories with the contact details of the department contact point (usually the on-plant officer or veterinarian) as part of their arrangements with the testing laboratory.

The same requirements apply to both independent and in-house laboratories.

## 5.6. Costs

Laboratories will be required to meet all cost associated with department approval that are not covered under the routine operation of the department. This may include the cost of the department on-site assessments and the processing of applications for approval.

Laboratories must meet all costs associated with third party accreditation and proficiency testing.

# 6. GENERAL RESPONSIBILITIES

## 6.1. Laboratories

To gain departmental approval a laboratory must demonstrate competence in the methods for which they are seeking approval. This will involve:

- Completion of a written application for approval to the department (for a copy of application form refer to Annex 1 of the [Approved Laboratory Program](#) document)
- Submission of laboratory manuals for all methods for which department approval is sought
- Provision of evidence of competency (e.g. NATA accreditation if applicable, internal proficiency results, training records, etc.) for each department approved method
- Agreement to meet laboratory requirements as specified in this manual (Section 5).
- Obtaining approval for all methods used for testing under the scope of the departmental lab approval.
- Must notify the department (Micro\_Program@aff.gov.au) of any changes in their scope of accreditation and management or any other changes that may reasonably be expected to impact on the competency of the laboratory.

## 6.2. Establishments

With respect to department microbiology monitoring programs, establishment management have responsibility to:

- Develop and implement a program compliant with the requirements of this manual and relevant to the establishment's export registration and incorporate this into their Approved Arrangement (AA)
- Ensure that personnel collecting and handling samples are competent to perform these functions and have been properly trained and monitored and that these activities are properly recorded and that these records are available to the department
- Ensure all testing is performed at a department approved laboratory using department approved methods
- Where appropriate, notify laboratories of testing and reporting requirements associated with particular samples
- Authorise and instruct testing laboratories to provide all relevant test results to department on-plant officers before or at the same time they are provided to establishment management
- Make and retain records of all results of screening tests, confirmatory tests and dispositions
- Support department verification activities undertaken to ensure programs are functioning correctly and efficiently
- Ensure that all training and verification records are maintained and available for review
- Ensure that a Request for Permit is not validated until all relevant final microbiological results relating specifically to product certification are known.

In the event of a result that indicates failure of a standard or market requirement, establishment management must ensure that:

- Implicated product is retained or can be traced and quarantined
- The department on-plant officer is notified without delay. Where the department officer is not present at the establishment, a copy of the notification must be e-mailed to Mid – Operations in Canberra, Attention: Laboratory Program at [MID.OpsCoord@aff.gov.au](mailto:MID.OpsCoord@aff.gov.au).

## 6.3. Sample Handling and Transport

The following requirements for the handling and transport of specimens apply across all testing programs in this manual. The temperature requirements specified are consistent with the current Australian Standard. Where samples are tested for APC, a temperature range of 0-5°C must be used. Where samples are not being tested for APC a wider acceptable temperature range can be used, i.e. 0-7°C.

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All relevant documentation pertaining to the samples must be sent with the samples to ensure adequate identification of samples and notification of testing requirements to the laboratory. This information must include the date of collection, and the time that the first sample was collected.

Samples must be transported in appropriate packaging and should maintain sample temperature during transport such that on receipt at laboratory, the temperature of the sample does not preclude its testing. Establishments should contact their laboratory for advice on the packaging of samples or follow the International Air Transport Association (IATA) or a similar packaging and despatch methodology.

### **Swab Samples**

- Carcase swab samples must be packed in such a manner as to maintain the sample temperatures described above during transport to the laboratory. The procedure for preparing samples for transport to achieve this outcome must be documented in the establishment's AA.
- Carcase or surface swabs must not be frozen for transport. If a delay in transport of the sample is expected, the carcasses should be put aside and sampled at a time when the transport time/temperature objectives can be met
- Samples should be dispatched on the day of collection and analysis commenced on the day following collection and no later than on the second day following collection
- Bags containing sample sponges should be firmly secured to prevent leakage.

### **Samples of fresh meat**

- Samples of fresh meat must be packed in such a manner as to maintain the sample temperatures described above during transport to the laboratory. The procedure for preparing samples for transport to achieve this outcome must be documented in the establishment's AA
- Samples should be dispatched on the day of collection and analysis commenced on the day following collection and no later than on the second day following collection
- Unless otherwise specified, unfrozen fresh tissue samples must not be frozen prior to or during transport.

### **Samples of frozen meat**

- Frozen meat samples can be maintained frozen for up to 7-days after collection. Frozen meat samples can be held frozen during transport or transported at 0-5°C/0-7°C to allow thawing during transportation. Frozen samples must not be re-frozen once thawed or transported at 0-5°C/0-7°C.

### **Samples arrive above specified temperatures**

- Where samples arrive at the laboratory at a temperature >7°C but ≤10°C, analysis for enteric bacteria (*E. coli*, *Salmonella*, etc.) can proceed but the laboratory must

contact the department and the establishment to determine if the results of analysis are meaningful. (Samples tested for APC must be between 0°C and 5°C)

- Analysis should not be carried out on samples that arrive at temperatures >10°C without written approval from the department
- In all cases where high temperature precludes analysis the laboratory must notify the department and establishment and a new sample provided.

### **Laboratory**

On arrival at the laboratory, laboratory personnel must:

- Verify the integrity and temperature of the sample
- Determine that analysis of the sample can commence on the day of arrival or no later than the day following receipt of the sample
- Notify the department and the establishment should the time and/or temperature requirements not be achieved.

### **Establishment management**

- If advised of a failure to control temperature during transportation, either due to loss of integrity, traceability or unacceptable temperature or time of arrival, establishments must notify the department immediately and put procedures in place to prevent further failures from occurring
- The department Central Office (Residues and Microbiological Policy, Export Standards Branch) will determine if analysis of the sample can proceed or if a new sample is required to be collected by the establishment
- If agreed that a new sample is required, the procedures for repeating sample collection should be initiated (by the establishment).

## **7. MICROBIOLOGICAL TESTING PROGRAMS FOR DETERMINING HYGIENIC PERFORMANCE**

### **7.1. National Carcase Microbiology Monitoring Program**

All export registered slaughtering establishments are required to participate in the National Carcase Microbiology Monitoring Program (NCMMP) (formerly known as ESAM) which requires Aerobic Plate Count (APC) and *E. coli* (process control verification), and *Salmonella* testing (pathogen reduction), to verify slaughtering and chilling operations. This testing is an integral part of an establishment's HACCP based Quality Assurance program. Ongoing adverse *E. coli* testing trends, and/or detection of *Salmonella* above acceptable limits may be indicative that an establishment's HACCP has failed at one or several critical control points which should be investigated and if required, necessary corrective action taken.

The program is a test and release program meaning tested carcasses are not required to be held pending availability of the test results. The general requirements outlined below must be followed and the results of testing must be reported in the PHI database in the Meat Export Data Collection System (MEDC) system and any trending in the data considered by the establishment and the department.

### **7.1.1. Scope**

All export registered slaughtering establishments slaughtering animals under the scope of relevant export legislation and export registered game processing establishments.

### **7.1.2. Selection of carcasses for sampling**

In most instances, the number of carcasses tested is proportional to the production volume. Carcasses from different shifts, slaughter chains, species and/or chillers must be sampled and tested independently based on the production volume for each shift, chain, species or chiller. Carcasses should also be selected randomly from those available for sampling.

One of the key elements of the microbiological testing is to assess and validate chiller performance. Therefore sampled carcasses should not be selected from one chiller only, but all chillers must be included in the sampling frame for the selection of sample carcasses.

Unless otherwise allowed, sampling frequencies as specified in 7.1.6 should be applied.

Where these sampling frequencies do not achieve a minimum of one test per day for indicators of process control i.e. *E. coli* and APC, establishments should seek to achieve this minimum level of testing.

### **7.1.3. Sampling location/time**

Carcasses are sampled according to the requirements identified in Table 1. The department recognises that the sampling time may vary from operation to operation, however the time of sampling should be consistent within an operation to the extent possible

Table 1: Required sampling time for different species

Species	Sampling time
Cattle, pigs, horses, mules, donkeys and camels	After a minimum of 12 hours active chilling
Sheep, goats, ratite and other small stocks	After a minimum of 4 hours active chilling

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Pre dressed skin on kangaroo	As soon as possible after arrival in either the receival area or the carcass holding chiller
Dressed wild game (kangaroo & wild boar)	After completion of dressing in the skinning room at a point immediately prior to exiting the processing floor
Hot boned/warm cutting carcasses	After completion of dressing at a point immediately prior to exiting the slaughter floor and following any final carcass wash or hot water decontamination treatment that may be employed
Hot bagged carcasses	Before bagging (i.e. similar to hot boned carcasses).

Where it is not possible to begin testing of samples within 24h of collection, selected carcasses should be appropriately retained for sampling at a more suitable time e.g. carcasses randomly selected on Friday afternoons, Saturdays, Sundays or public holidays.

#### 7.1.4. Sampling of Carcasses

For species other than wild game, separate carcasses or carcass sides must be selected for sampling for *Salmonella* and indicator organisms (*E. coli* and APC).

**Note:** In the case of wild game, the same swab can be used for all tests.

The number of samples required to be collected for a shift is based on the establishment's throughput for that shift. Sampling frequency is discussed in 7.1.6.

A sterile template defining the specific area to be swabbed should be used to aid in the collection of the sample. Commercially available disposable templates can be used, alternately clean templates can be sterilised by one of the following methods;

- Immersion in 82°C water for 10 seconds. Remove the template and air dry.
- Dipping into ethyl alcohol followed by flaming
- Wiping with 70% isopropyl alcohol and air dry, or
- Autoclaving at 121°C for 15 minutes.

Sterile templates should be placed on each of the identified carcass sampling sites and the site swabbed with a sponge. Templates must be sterilised between carcasses or a new sterile template used for each carcass.

Swabbing Materials used for sampling are:

Whirl-pack™. For further details about swabbing techniques, see Appendix 1.

**Note:** Other suitable sponges may be available and can be used as long as they are approved by the department.

The intent of this program is to sample carcasses at sites that represent the greatest level of contamination. Sites listed in this section for the different slaughter species

have been identified in the scientific literature to be the sites most likely to be contaminated during slaughter and dressing.

If there is evidence that these sites do not represent areas of likely contamination at individual establishments then alternate sites may be nominated by the establishment and agreed to by the department. Reasons for selecting alternate sites may include:

- Unavailability of sites due to dressing procedures at the establishment
- Treatment of the sites in such a way as to render them non-representative of the carcass as a whole i.e.
  - o Intensified targeted trimming of these sites
  - o Treatment of the site with an antimicrobial intervention (i.e. steam vacuum) that is not uniformly applied to the carcass surface.

#### 7.1.4.1. Large stock

Bovine (Cow/Bull & Steer/Heifer), Equine (Horses/Mules/Donkeys) and Camelidae

Sampling sites (S1, S2 and S3) for large animal carcasses are shown in Figure 1. Three sites each of 100 cm<sup>2</sup> must be sampled. Use one side of the sponge to sample the flank and brisket and use the other side to sample the butt.

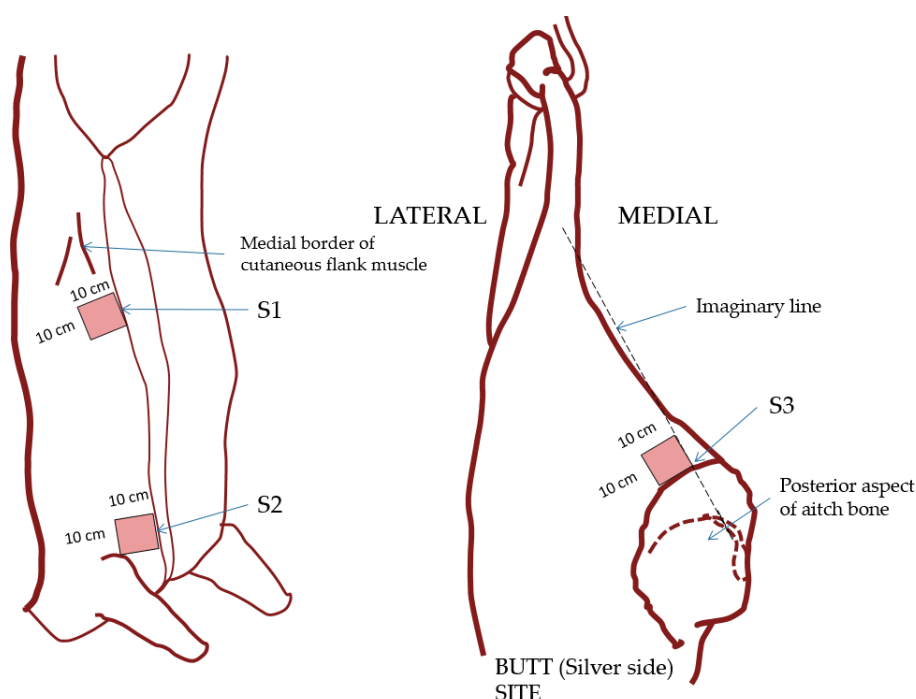


Figure 1: Sampling sites for large stock carcasses

For S1, locate the cutaneous flank muscle (external abdominal oblique) and follow the medial border of the muscle anteriorly until it comes approximately within 7.5 cm from the mid-line. Place your template and swab the area with a sponge starting from the midline.

For S2, locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut.

For S3, locate the posterior aspect of the aitch bone. Draw an imaginary line towards the Achilles tendon. At the point where the line intersects the cut surface of the round is the starting point for the butt sample. Measure 10 cm up the line leading to the Achilles tendon, then 10 cm over (laterally), then 10 cm back to the cut surface on the round, then 10 cm along the cut surface to form the 10 cm square area.

Samples are collected in the sequence of S1 first, then S2 and S3.

#### 7.1.4.2. Small stock

Ovine (sheep, lambs), Caprine, Calves and Cervine

Sampling sites (S1, S2 and S3) for small stock are shown in Figure 2. Three sites each of 25 cm<sup>2</sup> are sampled. Use one side of a sponge for flank and brisket and use the other side of the sponge for the mid-loin.

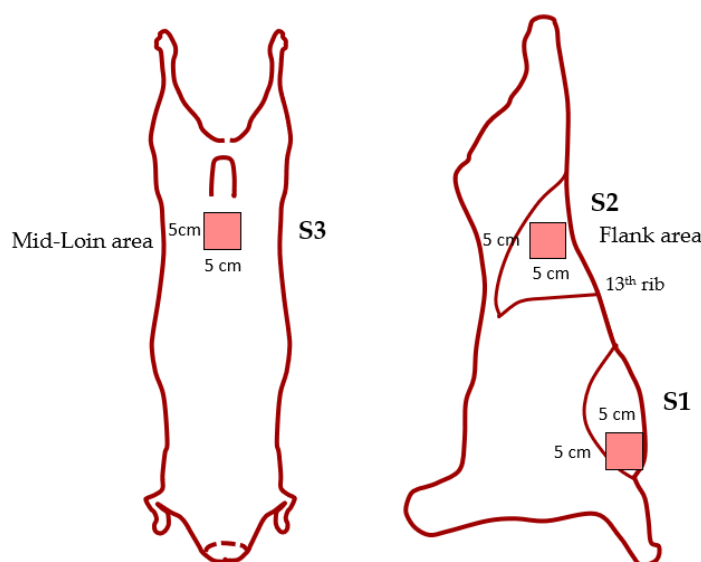


Figure 2: Sampling sites for small stock

For S1, Locate the elbow of the carcass. Draw an imaginary line straight across (medially) to the midline cut.

For S2, locate the caudal edge of the 13th rib. Place your template 7.5 cm above the caudal edge of the 13th rib going outside (laterodorsally).

For S3, locate the base of the tail. For sheep/lambs/ goats this is approximately 8 cm below the base of the tail, and for calves and deer approximately 12 cm below the base of the tail.

Samples are collected in the sequence of S2 first, then S1 and S3.

#### 7.1.4.3. Porcine

Sampling sites (S1, S2 and S3) for domestic pig carcasses are shown in Figure 3. Three sites each of 100 cm<sup>2</sup> are sampled. Use one side of a sponge for the belly and ham and use the other side of the sponge for the jowl.

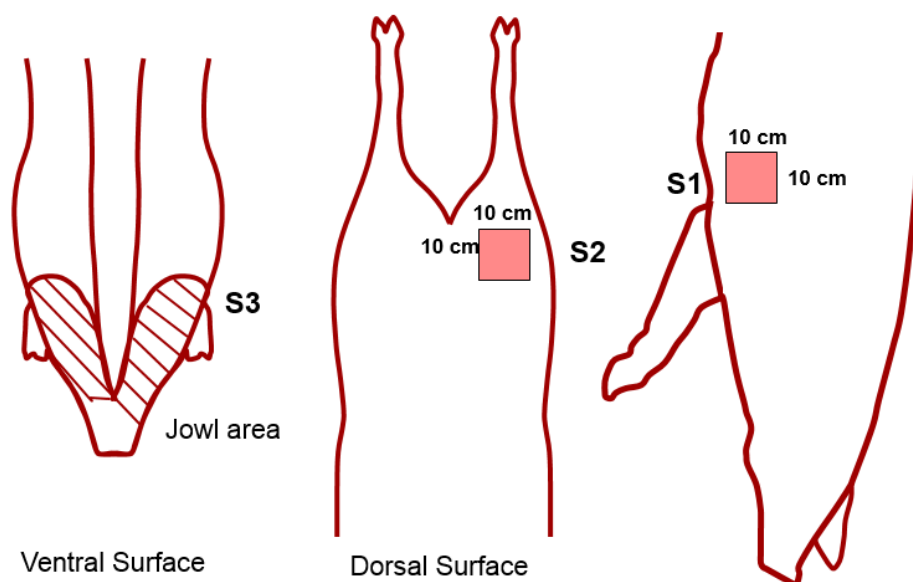


Figure 3: Sampling sites for porcine carcasses

For S1, locate the elbow of the carcass. Draw an imaginary straight line across (medially) to the midline cut.

For S2, from the dorsal position locate the lateral surface of the base of the tail and measure up (caudal) 5 cm along the lateral edge of the exposed fat margin.

For S3, draw an imaginary line from the atlas/axis joint to the ventral midline; all skin below that point will be considered the jowl.

Samples are collected in the sequence of S1 first, then S2 and S3.

#### 7.1.4.4. Ratites

Sampling sites for ratites are shown in Figure 4. Three sites each of 25 cm<sup>2</sup> are sampled. Use one side of the sponge for two sites and other side of the sponge for the third site.

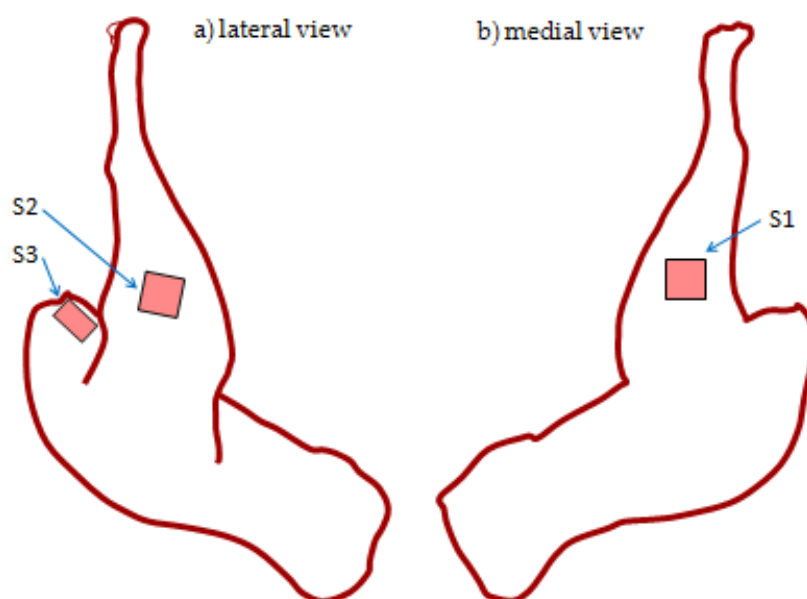


Figure 4: Sampling sites for ratite carcasses

S1, the medial surface of one leg, is at the innermost surface of the big drum muscle, centred on the line of the opening cut through the hide.

S2, the lateral surface of the same leg, is at a point defined by the outermost surface of the flat drum muscle.

S3, the leading surface of the pelvis, is on the long steak muscle as close as practically possible to the vent.

For carcasses processed using the single leg procedure, sampling should be conducted on the first leg.

#### 7.1.4.5. Macropods (Kangaroos)

Sampling sites (S1, S2 and S3) for kangaroo are shown in Figure 5.

- Where pre-dressed carcasses are required to be sampled, samples should be collected as soon as possible after arrival at the establishment. The lower edge of the template should be placed approximately 5 cm above the posterior edge of the kidneys.
- Dressed carcasses are sampled at three sites each of 25 cm<sup>2</sup>. Use one side of a sponge for shoulder and rump and use the other side of the sponge for the belly. If sampling carcasses pre-dressing i.e. on arrival at the establishment then sample the internal body cavity over the loin.

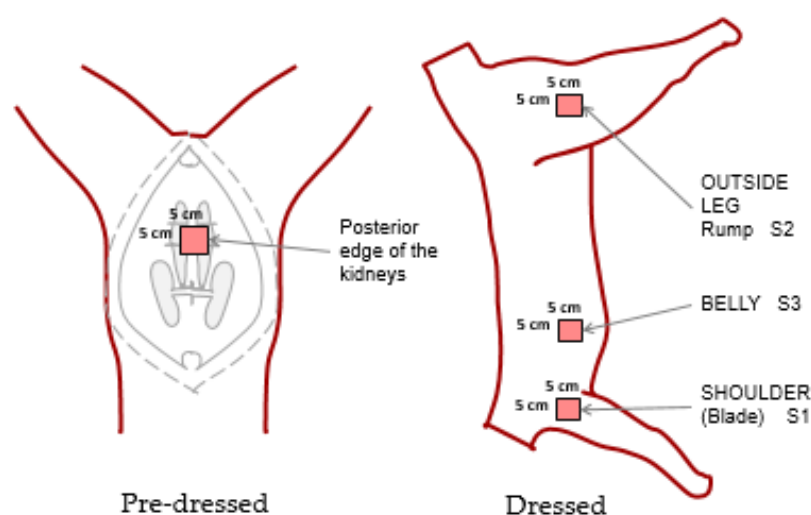


Figure 5: Sampling sites for macropod carcasses: Internal tenderloin fillet for pre-dressed carcasses, three sites for the dressed pre-boning carcase.

S1, at the shoulder (forequarter), is on the lateral surface of the carcase just in front (anterior) of the shoulder blade (the spine of the scapula).

S2, at the rump (hindquarter), is on the lateral surface of the carcase, half way between the stifle (knee) and the hip.

For S3, at the belly, locate the last thoracic rib. The site is in alignment with the caudal edge of the last thoracic rib and bordering the mid-line cut.

Individual pieces of **bulk packed manufacturing meat** are sampled by cutting a single 25 cm<sup>2</sup> slice of surface tissue (maximum thickness ~ 5 mm) from product with a sterile instrument. Equipment must be sterilised between cartons. The sample is placed aseptically into a sample container or a plastic dilution bag at the establishment and transported to the laboratory.

#### 7.1.4.6. Wild Boar

Sampling sites (S1 and S2) wild boar carcasses are shown in Figure 6. Two sites each of 25 cm<sup>2</sup> are sampled as the head/jowl is not available (not shown in figure). The belly site should be sampled first followed by the hind leg.

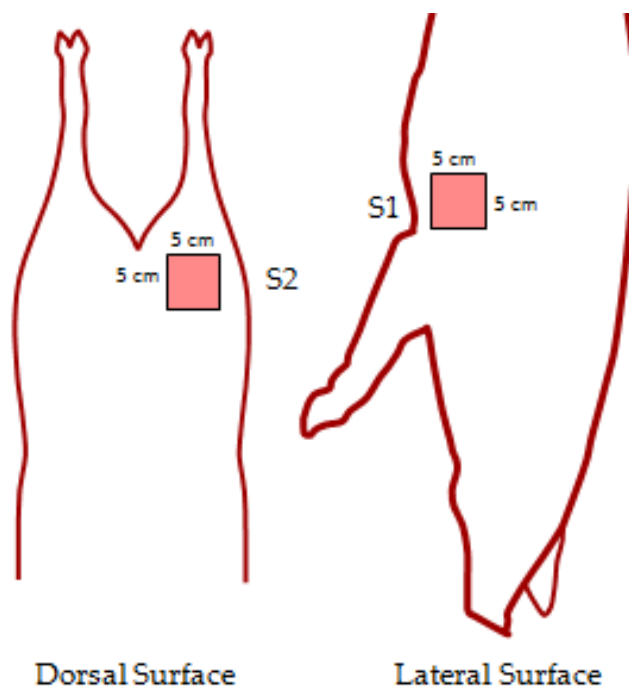


Figure 6: Sampling sites for wild boar carcasses

For S1, at the belly, locate the elbow of the carcass. Draw an imaginary straight line across (medially) to the midline cut. The edge of the site aligns with the midline cut and is bordered above by the imaginary line.

For S2, at the hind leg, from the dorsal position locate the lateral surface of the base of the tail, measure up (caudal) 5 cm along the lateral edge of the exposed fat margin (the starting point). The top left hand corner of the site aligns with the starting point.

### 7.1.5. Sampling Procedure

The recommended technique for the collection of swab samples is provided in Appendix 1.

### 7.1.6. Carcase Sampling Frequency

#### 7.1.6.1. *E. coli* and APC

Samples are taken at a frequency based on the volume of production. Minimum sampling rates for each slaughter class are provided in Table 2. The intention of these minimum rates is to ensure that at least one sample is collected daily at the establishment.

The sampling frequency must be determined separately for each slaughter class, chain, shift and for cattle carcasses that are hot boned. Analysis for generic *E. coli* and APC can be performed from the same swab.

Table 2: *E. coli* and APC sampling frequency and area for different species

<b>Class of Stock*</b>	<b>Sampling Frequency</b>	<b>Sampling Area</b>
Steer/Heifer	1 test per 300 carcasses	300 cm <sup>2</sup>
Cow/Bull	1 test per 300 carcasses	300 cm <sup>2</sup>
HB# cattle	1 test per 300 carcasses	300 cm <sup>2</sup>
Horse/Mule/Donkey	1 test per 300 carcasses	300 cm <sup>2</sup>
Camel	1 test per 300 carcasses	300 cm <sup>2</sup>
Pig	1 test per 1,000 carcasses	300 cm <sup>2</sup>
Sheep	1 test per 1,000 carcasses	75 cm <sup>2</sup>
Lamb	1 test per 1,000 carcasses	75 cm <sup>2</sup>
Calf	1 test per 1,000 carcasses	75 cm <sup>2</sup>
Goat	1 test per 1,000 carcasses	75 cm <sup>2</sup>
Deer	1 test per 1,000 carcasses	75 cm <sup>2</sup>
Pre-dressed kangaroo	1 test per 300 carcasses	25 cm <sup>2</sup>
Dressed kangaroo	1 test per 600 carcasses	75 cm <sup>2</sup>
Bulk-packed kangaroo	1 test per 500 cartons	25 cm <sup>2</sup> by 5mm deep
Dressed wild boar	1 test per 200 carcasses	50 cm <sup>2</sup>
Ratite	1 test per 3000 carcasses	75 cm <sup>2</sup>

\* Establishments should collect a minimum of one sample per day for indicators of process control i.e. *E. coli* and APC.

# Hot boned carcasses

#### 7.1.6.2. Salmonella

Samples shall be taken at a frequency based on a slaughter establishment's volume of production at the minimum rates detailed in Table 3.

Table 3: *Salmonella* sampling frequency for different species

Class of Stock	Sampling Frequency	Sampling Area
Steer/Heifer	1 test per 1500 carcasses	300 cm <sup>2</sup>
Cow/Bull	1 test per 1500 carcasses	300 cm <sup>2</sup>
HB cattle*	1 test per 1500 carcasses	300 cm <sup>2</sup>
Horse/Mule/Donkey	1 test per 1500 carcasses	300 cm <sup>2</sup>
Camel	1 test per 1500 carcasses	300 cm <sup>2</sup>
Pig	1 test per 5,000 carcasses	300 cm <sup>2</sup>
Sheep	1 test per 5,000 carcasses	75 cm <sup>2</sup>
Lamb	1 test per 5,000 carcasses	75 cm <sup>2</sup>
Calf	1 test per 5,000 carcasses	75 cm <sup>2</sup>
Goat	1 test per 5,000 carcasses	75 cm <sup>2</sup>
Deer	1 test per 5,000 carcasses	75 cm <sup>2</sup>
Dressed kangaroo <sup>#</sup>	1 test per 3000 carcasses	75 cm <sup>2</sup>
Dressed wild boar <sup>#</sup>	1 test per 1000 carcasses	50 cm <sup>2</sup>
Ratite	1 test in any week that ratites are processed	75 cm <sup>2</sup>

<sup>#</sup> Hot boned carcasses

<sup>#</sup> Analysis for *Salmonella* can be performed from the same sample as collected for *E. coli* and APC.

#### 7.1.7. Sample Analysis

##### 7.1.7.1. Laboratory requirements

All testing must be carried out at a department approved laboratory. All *E. coli* and *Salmonella* samples collected as part of the National Carcase Microbiology Monitoring Program must be tested in a NATA accredited laboratory. Laboratories will be approved subject to meeting the requirements of ISO/IEC ISO 17025 *General requirements for the competence of testing and calibration laboratories*.

Requirements of department approval are identified in Section 5 of this manual.

All methods used for the analysis of samples defined under this manual must be approved by the department for the specific purpose. A [list of approved methods](#) is available on the department website. Test methods have been approved based on

performance and ease of use as well as acceptability by markets (where this is relevant).

#### 7.1.7.2. Test methods

For APC and *E. coli* testing the total amount of diluent (buffered peptone water, peptone salt solution, Butterfields diluent or other approved diluent) added to the sponge must be 25mL. The sponge is palpated or stomached to ensure the sample is homogeneous. A portion of diluent is removed and analysed for APC and *E. coli* according to the approved procedure. If results for coliforms are generated voluntarily, they can be entered into MEDC if establishments choose. Results for coliforms do not contribute to PHI index score.

**Note:** The laboratory must be instructed by the establishment to analyse sufficient dilutions to ensure that where sufficient microorganisms are present, a count is obtained for each sample. The number of dilutions can be reduced once a history of the level of bacteria present is obtained and the laboratory is confident that the dilutions used will provide a numerical count.

Where counts are outside the countable range of the test being performed an estimate of the count must be reported. If counts are repeatedly outside the countable range, the laboratory should investigate to ensure appropriate dilutions are used in future.

For *Salmonella* samples the amount of diluent (BPW) in the final sample should be between 60-100 mL to ensure that the sponge is covered during enrichment.

#### 7.1.8. Reporting of Test Results

All test results with one or more typical colonies must be reported into MEDC, irrespective of the minimum acceptable level of contamination for that particular sample. A zero result (i.e. no colonies on plates) is reported as less than (<) the limit of detection of that method (this must include consideration of the dilutions used by the laboratory).

**Note:** For reporting into MEDC a zero count is reported as zero, not less than, the limit of detection. The above reporting requirements only apply to laboratory reports provided to the customer.

Laboratories must report the actual result i.e. less than the lower limit of detection, greater than the upper limit of detection or an actual number between these limits, as appropriate. Laboratories must ensure that a sufficient number of dilutions are tested to enable quantitative results to be reported down to the lower limit of detection for the method used to analyse the sample.

- Results for the National Carcase Microbiology Monitoring Program swab testing for generic *E. coli*, and APC on carcasses must be reported as CFU/cm<sup>2</sup> of carcase surface
- Coliform results, if generated, can be entered into MEDC on a voluntary basis. Results for carcase coliforms do not contribute to PHI index score.

- Surface excision samples of bulk packed primals or carcass parts (i.e. kangaroo meat) must be reported as CFU/cm<sup>2</sup>
- All samples tested for *Salmonella* are reported as 'Negative' or 'Positive' based on the test result being not detected or detected.

### 7.1.9. Calculation of Test Results

Examples of calculations of different type of samples are provided in Appendix 2.

### 7.1.10. Interpretation of Test Results

#### 7.1.10.1. Carcass *E. coli* and APC

Interpretation of results for carcass *E. coli* and APC<sup>2</sup> is based on a 'three-class sampling plan'. The performance of a three-class sampling plan is defined by a value at or below which the results are acceptable (*m*), a value above which the sample is unacceptable (*M*) and the number (*c*) of marginal samples (*>m* but *≤M*) in a defined sample window of (*n*) samples e.g.:

- Acceptable – less than or equal to the acceptable value (*≤ m*)
- Marginal – greater than *m* but not higher than the upper limit of acceptability *M* (*>m*, but *≤ M*)
- Unacceptable – greater than *M* (*>M*).

##### 7.1.10.1.1. Performance Standard - *E. coli* and APC on carcasses

The department has established target limits for generic *E. coli* and APC that are assessed on a moving window of 15 consecutive samples to allow for continuous evaluation of performance (Table 4). The latest result is compared to the previous 14 results (*n-1*) to determine if the performance standard has been met.

In order to allow for corrections in the process to be evaluated the window must be 'reset' after each failure and implementation of corrective and/or preventive action.

A window of *n=15* samples will fail if the number of marginal results (*>m* but *≤M*) exceeds '*c*'; or a single result exceeds *M*. Such results will trigger an 'ALERT'.

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<sup>2</sup> APC performance criteria apply to EU listed establishments only

Table 4: *E. coli* and APC performance criteria (PC) for carcasses of various species/categories sampled under this program

Species/ category	Window (n)	Marginal Results (c)		m <sup>#</sup> CFU/cm <sup>2</sup>		M CFU/cm <sup>2</sup>	
		<i>E. coli</i>	APC <sup>^</sup>	<i>E. coli</i>	APC <sup>^</sup>	<i>E. coli</i>	APC <sup>^</sup>
Steer/Heifer	15	3	3	0	1000	20	31625
Cow/Bull	15	3	3	0	1000	20	31625
HB* Cattle	15	7	3	0	1000	50	31625
Calf	15	7	3	5	1000	100	31625
Goat (skin-off)	15	3	3	1	1000	100	31625
Goat (skin-on)	15	3	3	1	1000	100	31625
Deer	15	7	3	1	1000	100	31625
Horse/Mule	15	5	3	0	1000	100	31625
Donkey	15	5	3	0	1000	100	31625
Camel	15	5	3	0	1000	100	31625
Sheep	15	7	5	5	1000	100	31625
Lamb	15	7	5	5	1000	100	31625
Pig (skin-off)	15	5	5	1	3162	100	31625
Pig (skin-on)	15	5	5	1	3162	100	31625
Ratite	15	3	3	1	1000	10	31625
Kangaroo (dressed carcasses and bulk packed)	15	7	4	50	10000	500	31625
Wild boar (dressed carcasses and bulk packed)	15	4	4	50	10000	500	31625

\* Hot boned carcasses

<sup>#</sup> Values of zero for “m” represent no detection using the sampling and testing protocols outlined in this manual i.e. a positive detection is considered a marginal result

<sup>^</sup> APC performance criteria apply to EU listed establishments only

An 'ALERT' will require the establishment to initiate a review of the carcase dressing procedures and conduct an investigation into the possible contributing causative factors within ten working days of *E. coli* window failure. It also should include corrective and preventative action undertaken to prevent the recurrence of the contributing factors. These may include:

- External factors such as harvesting, lairage, stock condition, etc.
- Internal factors such as carcase dressing procedures, employee training, refrigeration practices, etc.
- Review outcomes should be documented and signed by the person(s) responsible for the action and be available to the department for audit purposes
- Verification of corrective and preventive action is to be achieved through further monitoring under this program.

#### **7.1.10.2. Carcase Salmonella Testing**

Carcases of all slaughter classes are tested for *Salmonella* at the frequency specified in Table 3. Results are assessed against *Salmonella* performance standards as discussed in the following paragraphs. Where a sample tests positive for *Salmonella*, it must be serotyped at a *Salmonella* reference laboratory (see 7.1.10.2.3).

##### **7.1.10.2.1. Salmonella Performance Standards**

The positive detection of *Salmonella* must be assessed against the performance standards described in Table 5. A positive *Salmonella* test result will trigger a sample-window. The 'window' will be representative for the relevant class of product. It will require the establishment to immediately commence daily sampling until a window is satisfactorily completed for that slaughter class. Any further *Salmonella* positives that exceed the acceptable number of positives within the same sample window would result in failure of the window. For example in the Cow/Bull category, two *Salmonella* positives are permitted in a set of 58 samples, which constitutes a 'window'. Where the *Salmonella* detections are over the permitted number of positives, it will be classed as 'Failure of the Window'. In such cases, stop further sampling irrespective of whether or not the number of samples required to complete that sample window are achieved - it is not necessary to complete the window.

For game pig and kangaroo, if a positive *Salmonella* detection occurs, normal sampling is to continue at the rate in section 7.1.6.2 and a sample window commences from the positive sample identification (i.e. the positive is included in the sample window). In the event that a second positive *Salmonella* detection occurs within the same window of 55 samples, it will be deemed that the 'window has failed'. The establishment must investigate to determine the cause of the non-compliance and implement effective corrective/preventive action. The effectiveness of the corrective/preventive action must be verified through enhanced levels of oversight and audit. In the event a processing deviation could not account for the findings, external factors (e.g. collection, storage and transportation) should be investigated. Where *Salmonella*

detections exceed the permitted number of positives, sampling is to stop irrespective of whether or not the number of samples required to complete the sample window are achieved and a new window of 55 samples must commence once corrective actions have been taken.

Table 5: *Salmonella* performance standard for carcasses of various species/categories

Slaughter class	Number (n) of samples in a window	Number (c) of allowed positive results #
Steer/Heifer	82	1
Cow/Bull	58	2
Horse/Mule/Donkey	58	2
Camel	58	2
Skin-on and skin-off pig, sheep, lamb, calf and deer	55	6
Skin-on & skin-off goat	51	12
Kangaroo	55	1
Wild Boar	55	1
Ratite	55	2

# The maximum number of positives acceptable in a window of n samples based on national prevalence data for *Salmonella* on carcasses from the indicated species

#### 7.1.10.2.2. Failure to meet *Salmonella* Performance Standard

The establishment, within 10 working days must investigate possible causes of any failure to meet the *Salmonella* performance standard. Should evidence of poor hygiene or sanitary dressing be obtained, corrective and preventive action must be taken. The effectiveness of corrective action is to be verified through increased oversight and audits. In the event a processing deviation does not account for the findings, pre-slaughter factors, e.g. cleanliness of livestock and transport or animal stress (including feeding practices), should be investigated.

Once the establishment has completed an investigation and implemented corrective actions that have been verified by the department as satisfactory, the establishment will commence a second window as per the performance criteria specified in Table 5.

If the establishment fails to meet the performance standard on the second window for that class of product, the establishment shall re-assess its HACCP plan for that product and take appropriate corrective action. The establishment will start the third sample window

Failure of a third consecutive *Salmonella* window coupled with an unsatisfactory establishment investigation and corrective action will result in a Critical Incident Reponse Audit as detailed in the [Critical Incident Response Guidelines](#).

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#### **7.1.10.2.3. Salmonella serotyping**

All *Salmonella* isolates obtained under this program must be sent to a *Salmonella* reference laboratory for typing. Results of typing must be reported to the department once received by the testing laboratory. The contact details for reference laboratories are available from the department by emailing Micro\_Program@aff.gov.au.

#### **7.1.11. Wild Game Pre-dressing Microbiology**

Pre-dressing sampling results are to be recorded on-plant and used by processors as an additional verification of field dressing hygiene and cold chain practices. Acceptance criteria should be developed by the establishment and unacceptable results should be used as a tool to improve harvester/field depot practices. Records of results and any actions taken must be provided to the department for audit purposes.

### **7.2. National Carton Meat Microbiology Testing Program**

All establishments producing carton or bulk packed meat for export must collect and analyse tissue samples from final product for APC. This includes all game establishments. The procedure for collecting and analysing samples must be included in the establishment's AA. Test results for APC must be reported in the PHI database in MEDC. If results for coliforms are generated voluntarily, they can be entered into MEDC if establishments choose. Results for coliforms do not contribute to PHI index score.

#### **7.2.1. Selection of cartons for sampling**

Samples must be collected as close to final carton closure as possible. Where boned product is produced in other than a carton (e.g. production of 'combos' for export), equivalent arrangements with regards to the testing of final product must be undertaken and approved by the department. When selecting cartons for sampling the following conditions apply:

- Cartons from different shifts, boning and/or species must be sampled and tested independently
- Cartons must be selected randomly from those available for testing as defined above.

#### **7.2.2. Sampling procedure and frequency**

- The intent of this program is that each slaughter establishment, independent boning room or wild game processing establishment should be collecting at least one sample per day
- At slaughter establishments, samples are to be collected from carton meat (bulk packed trim or similar product) at the same frequency as APC/*E. coli* carcase samples i.e. if three carcase APC/*E. coli* samples are collected daily then three carton meat samples should be collected and analysed daily as well

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- Where an establishment is an independent boning room and does not conduct carcass swabbing then carton samples must be collected according to the carcass equivalents processed daily. For the purpose of this program carcass equivalents are defined as follows:
  - For bovines: Eight cartons are assumed to be equivalent to one large stock carcass and the NCMMP sampling rate for bovines is one swab per 300 carcasses. Therefore, one carton should be sampled for every 2400 cartons produced. Where establishments produce multiples of 2400 cartons of beef per day, e.g. 7200 cartons, they are required to collect three samples for that day; and
  - For ovines: one carton is assumed to be equivalent to one small stock carcass and the NCMMP sampling rate for sheep is one swab per 1000 carcasses. Therefore, if producing 1000 cartons of sheep meat a day the requirement is to collect one carton meat sample per day
- Sampling is by surface slices or small grab pieces of meat originating from the surface of the carcass and weighing approximately 5-10 g. The pieces of meat are selected such that a minimum of five 5-10 g pieces are collected from individual cartons. Where this is not practical, i.e. trim is not produced on the day, whole muscle product should be sampled. In this case a surface slice weighing approximately 25 g and no more than 5 mm thick is to be collected and analysed.

### **7.2.3. Sample Analysis**

#### **7.2.3.1. Laboratory requirements**

All testing must be carried out at a department approved laboratory. Requirements of department approval are identified under Section 5 of this manual.

#### **7.2.3.2. Test methods**

All methods used for the analysis of samples defined under this manual must be approved by the department for the specific purpose. A list of [approved methods](#) is available on the department website. Test methods have been approved based on performance and ease of use as well as acceptability by markets (where this is relevant).

#### **7.2.3.3. Testing requirements**

A 25 g test sample is homogenised in 225 mL of diluent (i.e. BPW, peptone salt solution, Butterfield's diluent or similar diluent as recommended in the method) and analysed for APC. The laboratory must be instructed to analyse sufficient dilutions to ensure that a count is obtained for each sample. The number of dilutions used can be reduced once a history of results is obtained and the laboratory is confident that the dilutions used will provide a count. Where counts are outside the countable range of

the test being performed an estimate of the count (where obtainable) is to be reported.

#### **7.2.3.4. Calculation of Test Results**

Carton sample results must be reported as CFU/g of meat. Examples of calculations of different type of samples are provided in Appendix 2.

#### **7.2.3.5. Reporting of Test Results**

Results for carton testing (including all country specific testing) must be reported to the on-plant supervisor (i.e. on-plant veterinarian) at the same time that they are reported to plant management. All results must be captured nationally in MEDC.

### **7.3. Interpretation of Test Results**

Interpretation of results is based on national tercile values obtained from industry norms. These values are applied in the PHI.

## **7.4. Microbiological Testing of Ready-To-Eat meat products and Processing Environments**

### **7.4.1. Background**

*Listeria monocytogenes* is found throughout the environment and has been isolated from domestic and wild animals, birds, soil, vegetation, fodder, water and from floors, drains and wet areas of food processing establishments. It can cause illness in people when consumed in large numbers especially when consumed by pregnant women, the very young, the elderly, and immunocompromised. Businesses producing RTE meat products are required to manage food safety hazards such as *Listeria* and validate their processes for manufacturing packaged RTE meats. They must be licensed with the relevant state government food safety agency and manufacture product in accordance with the *Australia New Zealand Food Standards Code* (the Food Standards Code).

The Food Standards Code: Standard 1.6.1: "Microbiological Limits for Food" identifies RTE foods and the microbiological criteria that apply. The Food Standards code should be read in conjunction with the relevant Australian Meat Standard and guideline documents provided by FSANZ or the Meat Standards Committee.

### **7.4.2. Scope**

This section applies to ALL export registered establishments producing packaged ready-to-eat (RTE) meat and poultry products. Specific country requirements are summarised in Section 8.4.

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### 7.4.3. Purpose

For the purpose of this manual a RTE meat product is defined as a product that is edible without additional preparation to achieve food safety but may receive additional preparation for palatability or aesthetic, epicurean, gastronomic or culinary purposes.

This manual summarises the Meat Standards Committee “Regulatory guidelines for the control of *Listeria*” and specific requirements of the Food Standards Code. They do not replace this manual and should not be applied without consideration of the MSC guidelines and the Food Standards Code.

Only department approved methods can be used to test environment and product samples described in this manual.

### 7.4.4. Definitions

For the purposes of this Section:

**Antimicrobial agent** is defined as a substance in or added to a RTE product that has the effect of controlling, reducing or eliminating a microorganism, including pathogens such as *L. monocytogenes*. To be considered effective against *L. monocytogenes* an antimicrobial agent should allow no more than a 2 log<sub>10</sub> (100 fold) increase in numbers of *L. monocytogenes* over the shelf-life of the product.

**Antimicrobial Process** is an operation, such as freezing or drying, which is applied to a RTE product that has the effect of suppressing or limiting the growth of pathogens.

**Lethality Treatment** is a process or treatment that eliminates or reduces the number of pathogenic microorganisms on or in a RTE product to a level that is considered safe. Levels that are considered safe for human consumption are those that meet the microbiological criteria specified in the *Australia New Zealand Food Standards Code*.

**Post-lethality processing environment** is the area in an establishment into which or through which product is moved, after having been subjected to a lethality treatment.

**Post-lethality exposed product** is unpackaged RTE product that comes into direct contact with untreated product or a food contact surface in the post-lethality processing environment.

**Post-lethality Treatment** is a lethality treatment that is applied to the product or becomes effective after post-lethality exposure. A post-lethality treatment is considered to be effective if it can be shown to achieve at least a one-log<sub>10</sub> (10-fold) reduction of *L. monocytogenes*.

**Ready-to-eat** in relation to meat products means products that are ordinarily consumed in the same state as that in which they are sold and do not require further processing (such as cooking), but may be defrosted, reheated or portioned before consumption.

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#### 7.4.5. Environmental Monitoring for *Listeria* spp.

All RTE export establishments must test their processing environment for the presence of *Listeria* spp. It has been shown that environmental monitoring in conjunction with product testing is the most effective way to control *Listeria* on product. This monitoring is not a replacement for normal contact surface monitoring that is carried out to verify cleaning at the establishment.

##### 7.4.5.1. Selection of sample sites

The choice of sites must be justified and documented in the establishment's AA.

**Zone 1** sites are those typically contaminated with *Listeria* while **Zone 2** sites are those that are known to harbour *Listeria* in the RTE processing environment.

Table 6: Classification of environmental sampling sites as part of a *Listeria* sampling plan (MSC Guideline, 2008)

Priority	Examples of sampling sites
Zone 1	Equipment that comes into direct contact with product after the lethality treatment (e.g. slicers, dicers, tables, etc.)
Zone 2	Floors, walls, ceilings, drain outlets, pools of water (e.g. on the floors of a manufacturing area or cold room), chiller doors, switches, floor joints/crevices

A minimum of five environmental sites must be sampled for *Listeria* spp. monthly (i.e. four Zone 1 and one Zone 2 site). Testing should include both samples collected before operations commence and samples collected during operation. The surface area swabbed should be  $\geq 30 \text{ cm}^2$  if possible.

In the event of a positive detection on a Zone 1 or Zone 2 surface the establishment must immediately investigate the possible cause of the problem and initiate corrective action in accordance with their AA. Further, the establishment must increase the frequency of environmental monitoring to weekly until three consecutive negative samples for the implicated area have been obtained.

In addition, where a Zone 1 positive result is obtained the isolate must be identified to species level. If the species detected is *L. monocytogenes* then the establishment must test and hold product batches from the day of the first Zone 1 positive result. The establishment must test all implicated batches for *L. monocytogenes* until the site has been cleared. Product batches are to be tested by analysing five samples each of 25 g per batch. Composite testing of these five samples is permitted at the laboratory provided compositing is allowed in the method used.

#### 7.4.6. Product Testing for *Listeria monocytogenes*

Finished packaged RTE products must be tested to verify good manufacturing practices. Packaged RTE products must be sampled at a minimum of once every three months.

- Where the establishment produces various types of RTE products on a single production line only one sample of product needs to be submitted for analysis
- Where RTE products are produced on various production lines, a sample of each product type (representing each production line) shall be submitted.

Unless otherwise specified for particular markets the following conditions shall apply;

- Department approved methods must be used for the analysis of samples
- A minimum of 25g of product must be analysed
- Quantitative determinations should be carried out on foods that do not support the growth of *Listeria* spp.

**Note:** Peer-reviewed predictive models may be used to determine if growth of *Listeria* spp. is likely to occur during the shelf-life of the product.

##### 7.4.6.1. Positive results

The department and the applicable State Food Authority must be notified within 24h of a RTE product testing positive for *L. monocytogenes*.

**Note:** RTE products that test positive for *L. monocytogenes* but do not support its growth may be eligible for release to markets that recognise a limit of <100 *L. monocytogenes* per g of product, at the time of consumption.

All product implicated by the positive result must be retained/recalled by the company. A test and hold clearance program must be implemented for batches of RTE produced on the implicated line until acceptable results are obtained for three consecutive batches

On obtaining a positive RTE result the establishment should undertake a thorough review of its *Listeria* control program and initiate appropriate corrective actions.

##### 7.4.6.2. Microbiological criteria for *Listeria monocytogenes* in RTE meat products

The microbiological criteria for *Listeria* in ready-to-eat foods in the Food Standards Code are summarised in Table 7 below.

Table 7: Excerpt from Schedule of Standard 1.6.1

Food	Microorganism	n	c	m
Ready-to-eat food in which growth of <i>Listeria monocytogenes</i> will not occur	<i>Listeria monocytogenes</i>	5	0	100 cfu/g
Ready-to-eat food in	<i>Listeria</i>	5	0	not

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which growth of <i>Listeria monocytogenes</i> can occur	<i>monocytogenes</i>			detected in 25 g
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A “lot” of a food fails to comply with the Standard if the number of defective sample units is greater than c (i.e. any positives detected) and the level of *Listeria monocytogenes* in a food in any one of the sample units is more than m.

#### 7.4.6.3. Validation

Establishments producing RTE product are required to manage food safety hazards and validate their processes for the manufacture of RTE foods. If applicable, validation documentation should provide the objective evidence that shows that the product does not support the growth of *L. monocytogenes* or that growth is limited (<100 cfu/g) under reasonably foreseeable conditions of distribution, storage, retail and use.

Validation information/documentation should be kept for each procedure and should be reviewed as required to ensure that it is current and continues to validate the process.

#### 7.4.6.4. Verification - Product Sampling and Analysis

Testing of finished, packaged RTE products is used to verify good manufacturing practices and should be conducted at a frequency to show the effectiveness of the establishment control of *Listeria*. The Meat Standards Committee “Regulatory Guidelines for the control of *Listeria*” requires the establishment to test more frequently when implementing a new process. They may then reduce testing frequency depending on the effectiveness of the process in controlling *Listeria*.

Prior to submitting samples for analysis, consideration needs to be given to which limit should apply and, therefore, whether enrichment (detection) and/or a quantitative method is to be used; as well as the time taken for testing (which may have implications for when a lot is released to the market). Food businesses must develop procedures for sampling and analysis, including details on any hold and test procedures and corrective actions and incorporate these into their AA.

A guidance document (“Guidance on the application of microbiological criteria for *Listeria monocytogenes* in RTE food”) providing information on the interpretation of the Food Standards Code in relation to *L. monocytogenes* may be found on the [FSANZ website](#).

## 8. MICROBIOLOGICAL TESTING TO SATISFY SPECIFIC MARKET REQUIREMENTS

This section provides a summary of known microbiological testing requirements implemented by particular export markets at the time of writing. The programs are

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undertaken at establishments with specific market listing that are producing, or intend to produce, product covered by the programs detailed below. They should not be used as a definitive reference for all testing requirements for all markets. Establishments must ensure that they are compliant with market requirements prior to exporting using the department's Manual of Importing Country Requirements (Micor) or other departmental references as required (e.g. market access advices, meat notices, etc.).

## **8.1. Shiga toxin-producing *Escherichia coli* testing of raw ground beef components intended for export to Canada, the United States of America and its territories**

This is a test and hold program designed to satisfy the requirements to export raw ground beef components to the USA and Canada and other markets where Shiga toxin-producing *E. coli* (STEC) testing is a requirement of export. Product must remain under the control of the establishment (able to be recalled) until the result of any testing under this program is known to be negative. All confirmed positive or deemed positive results must be notified to the department and a disposition applied.

### **8.1.1. Purpose**

To advise Australian export registered slaughter, boning establishments and independent cold stores listed to export raw ground beef components to the USA (USBG) and/or Canada (CABG) of the:

- Pre-export requirements to test raw ground beef components for *E. coli* O157 and if applicable, six additional STEC serotypes
- USA and Canada port-of-entry testing requirements for STEC testing.

The program defined in this section has been written to include testing for STEC serotypes to ensure compliance with USA and Canadian requirements.

Note: Specific serotypes targeted may vary between countries

### **8.1.2. Scope**

All Australian export registered establishments with single or dual listing to produce raw ground beef components that are intended for export or are exported to Canada, the USA or its territories.

### **8.1.3. Definitions**

For the purposes of section 8.1 of the manual, the following definitions apply:

**Confirmed Positive:** A test result indicating that an isolate obtained from a potential positive sample has been purified and confirmed as one of the seven STEC identified in this manual.

**Deemed Positive:** A sample that has returned a potential or presumptive positive result and has not been tested further to confirm the testing result as “test positive” or “test negative”. Product exported without testing when testing was required, or product found to be potential positive but where confirmation testing was not conducted, will be deemed positive for the purposes of disposition.

***E. coli* 0157:** An organism which gives a positive test for detection of *E. coli* serotype 0157 from an enrichment broth, and a pure isolate from the enrichment broth is:

- Confirmed with biochemical and serological tests as *E. coli* 0157, and
- Confirmed to contain one or more of the Shiga toxin genes (*stx1/2*) and the *eae* gene.

**Lot:** All cartons, packages, or containers represented by the tested sample and identified by the establishment as microbiologically independent from other lots based on a scientific, statistically based sampling program. This program should consider the possible connections between lots including lots produced from the same source material (source lots) and must:

- Not exceed 700 cartons (or equivalent)
- Be sampled using a robust n=60 sampling plan as described in this Section
- Only originate from a single packing establishment
- Be identified with a single port mark (or equivalent as defined in the AA)
- Not be redefined after sampling and testing
- Must not be retested to change the disposition of the lot
- Only be loaded in a single shipping container.

**Microbiological independence:** For the purpose of this program, the microbiological independence of a sampled lot is an indication of the separateness of one sampled and tested lot from another. In determining the independence of a lot the establishment should consider the formation of the lot, the robustness of the sampling regime(s) applied to the lot and other factors that assist in identifying one lot as distinct from another. It is the responsibility of the establishment to identify in the establishment's AA how the microbiological independence of a sampled lot is determined and maintained.

**Potential positive:** A positive result for a screening test for STEC.

**Raw ground beef components:** Raw ground beef components include all beef and veal bulk packed manufacturing trimmings and other beef and veal components such as primal cuts, sub primal cuts, head meat, cheek meat, oesophagus meat, heart meat and advanced meat recovery product intended for grinding in the USA and/or Canada.

**Shiga toxin-producing *E. coli* (STEC):** For the purpose of this section and USA and Canadian market access requirements, STEC comprise serotypes O157, O26, O45, O103, O111, O121 and O145. The organism isolated from an enrichment broth must be:

- Confirmed with biochemical and serological tests as *E. coli* O157, O26, O45, O103, O111, O121 or O145, and
- Confirmed to contain one or more of the Shiga toxin genes (*stx1/2*) and the *eae* gene.

#### 8.1.4. Lot identification and traceability

When a lot has been defined, the establishment must allocate a unique test lot identifier to all cartons, pallets or other containers forming the lot to ensure that the lot identity can be maintained, controlled and traced at all times.

When a lot is placed into a container as a sampled lot and tests negative for export to the USA or Canada:

- The sampled lot must be exported in its entirety and in a single container

**Note:** Where a sampled lot cannot be exported in its entirety that part of the lot that is not in the first container is no longer eligible for export to the USA or Canada and further testing will not make it eligible for export to that market.

- The lot must be identifiable to an individual shipping mark (port mark)
- More than one sampled lot can be shipped under a single shipping mark, but a sampled lot must not be split between shipping marks
- There must be traceability from the test result to the sample, to the sampled lot identifier and to the shipping mark
- The establishment is responsible for maintaining control of all sampled lots that test negative and that are eligible for export to Canada or the US or its territories until that sampled lot is presented at port-of-entry for inspection by the importing country competent authority. Raw ground beef component consignments transferred between registered export establishments must have their USA /Canada STEC test status referenced on the meat transfer certificate (MTC) and be able to be linked to the MTC. The MTC endorsement requirements for different STEC test statuses are given in Table 8.

Table 8: MTC endorsement requirements for various STEC test statuses for raw ground beef component exports to USA/Canada.

Sampled	Test status	USA/Canada eligibility	MTC endorsement
Yes	Negative	Eligible	Eligible for the USA and Canada
Yes	Result not yet available	Eligible pending test result	USA and Canada eligible subject to receipt of STEC test result
No	Not applicable	Eligible subject to testing	USA and Canada eligible subject to testing
Yes	Positive (deemed positive)	Eligible pending heat treatment in Australia	Product retained pending heat treatment
No - Intention to export to the USA or Canada abandoned	Not applicable	Ineligible	Not eligible for the USA and Canada
No – Ineligible for other reasons	Not applicable	Ineligible	Not eligible for the USA and Canada
No – Intention to export abandoned for all markets	Not applicable	Not applicable	Not applicable – no MTC required

#### 8.1.5. HACCP reassessment covering STEC control

To maintain eligibility for the USA and/or Canadian market for raw ground beef component exports, establishments must consider the potential hazard of STECs in their HACCP plans.

Establishments currently not listed for exporting raw ground beef components to the USA and /or Canada, will have to reassess and validate their HACCP plans before starting or recommencing exports of raw ground beef components to the USA and/or Canada. In these cases, the department will request the establishment to undertake enhanced verification testing (a minimum of 15 verification tests) of the establishment product before the establishment starts or recommences exporting raw ground beef components to the USA and Canada.

It is recommended that as well as the use of observation, the assessment includes microbiological testing of the process steps that occur during carcass dressing and finished product testing. Testing during the process may assist in the detection of the sources of potential contamination that may not be obvious. It is acceptable to use indicator organisms for this analysis of process.

The validation of the system of process control must include testing of finished product for STECs to show the system's capability. Testing of end product alone may be acceptable to validate the process if sufficient tests are undertaken to cover variations in incoming stock and seasonality.

Records must be kept of the rationale and data supporting the reassessment.

The HACCP reassessment should identify the predominant STEC serotypes identified in the classes of stock being processed. The HACCP reassessment must determine which STECs will be used to verify process control and used in robust testing of lots. Whether or not testing for the non-O157 STECs continues in loads of raw ground beef components intended for export to the USA and Canada depends on the outcome of the HACCP reassessment.

Following reassessment, establishments must continue to test for *E. coli* O157 as a minimum in all lots of raw ground beef components destined for the USA and Canada.

### **8.1.6. Sampling**

General considerations for sampling include:

- It is acceptable for selected cartons to be removed from production and stored under appropriate conditions for later sample collection; however these cartons form part of and must be returned to the sampled lot
- Samples collected over a number of production days must be stored frozen until pooled for analysis
- Where samples are collected at independent cold stores, lots may only consist of product from a single source boning establishment.

#### **8.1.6.1. Sample collection**

Samples are collected from a lot based on the establishment's sampling program, defined in the establishment AA. Sampling may occur either at the time of production or at the time of consolidation of lots for export. Where production based the lot must be clearly identified during the production run.

The establishment must ensure that the full range of the raw beef components intended for grinding in the USA and Canada have an equal opportunity to be sampled in the lot. The sample collected for this program is comprised of at least 60 sub samples (n=60) which must satisfy the following criteria:

- A sample must consist of at least 60 small pieces of meat (surface slices or grab samples) representing the surface of the carcass and weighing between 5-10g
- The pieces of meat are to be selected from a minimum of 12 cartons representing the sampled lot with a minimum of five pieces taken from each carton. Where the lot is less than 12 cartons in size, then all cartons must be sampled and the total number of sub-samples collected from these cartons must equal 60

- The total number of pieces sampled per lot must be at least 60 (i.e. N=60 sampling)
- The total sample weight collected must be at least 375g ± 37.5g
- The sample must be collected using sanitised instruments under sanitary conditions.
- For sample collection from frozen trimmings, samples must be collected from the surface of cartons (e.g. using a spade drill bit or chisel) to a maximum depth of 3 mm.
- Samples collected from frozen cartons must be kept frozen until dispatched to the laboratory for testing.

#### **8.1.6.2. Sample labelling**

The sample must be labelled appropriately to ensure the following information aligns with the information provided to the laboratory and appearing on the laboratory report:

- Establishment number (if samples are to be sent to an external laboratory)
- Date of sampling
- Packing line (if applicable)
- Unique identifier of the sampled lot
- Product description
- The name of the approved testing laboratory.

#### **8.1.6.3. Sample storage**

Analysis of samples must commence on or before the second day following sample collection. When testing is not going to commence immediately samples must be held under refrigeration (0°C to ≤7°C).

When sample analysis cannot commence on or before the second day following sample collection, samples must be frozen at the establishment immediately after collection and held frozen until transported to the laboratory for testing.

Samples collected from frozen cartons can be kept frozen until dispatched to the laboratory for testing. Frozen samples once thawed cannot be re-frozen.

#### **8.1.6.4. Sample dispatch**

Where samples are to be transferred to an off-site laboratory for analysis standard procedures for transport must be followed (see Section 6.3). The temperature of samples that have thawed during transport must be ≤7°C upon arrival at the laboratory (see section 6.3 Samples of frozen meat).

#### 8.1.6.5. Sample Analysis

Samples may only be tested in a department approved laboratory and analysed using department approved methods (refer to Section 5). [Laboratories approved](#) to test for STEC to support certification of exported raw ground beef components are identified on the departmental website along with the [approved test methods](#) used by that laboratory.

All samples returning a positive screening test must either undergo further confirmatory testing in a department approved confirmation laboratory or the product will be treated as a confirmed positive (deemed positive) lot.

All confirmation of potential positive samples must be carried out on a sample of the same enrichment broth from which the potential positive was identified. It is strongly recommended that the enriched broth is to be analysed for confirmation as soon as possible. Where immediate analysis is not possible, the enriched broth may be stored under refrigeration for up to seven days prior to confirmation.

Confirmation must include an immune-magnetic separation (IMS) step for the isolation of target STEC serotypes. A confirmed positive is reported when at least one colony (an isolate) conforms to the definition of STEC or *E. coli* O157 in this document.

Laboratories should consult the appropriate regulations when transporting “screen-test positive” enrichment or “screen-test positive” samples (see [Australian Government, Department of Infrastructure and Transport](#) or [National Transport Commission](#)).

#### 8.1.6.6. Sampled Lot Retention

This is a test and hold program, so the sampled lot must be maintained under the control of the establishment (i.e. able to be recalled) pending the results of the screening test or any confirmatory test.

In the event that a lot is confirmed as being positive or deemed positive for STEC, the lot must be retained and placed under department retention until a disposition is applied.

#### 8.1.7. Department Verification Testing

All establishments with USA and Canada listing for the production of export raw ground beef components must participate in the departmental verification testing program.

Where establishments with such a listing choose not to retain that listing or do not wish to participate in departmental verification testing, they:

- Must amend their AA to reflect this; and
- Must relinquish their listing for access to the USA and Canadian market for raw ground beef or raw ground beef components.

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Where an establishment has not previously exported raw ground beef components to the USA or Canada, that establishment must verify the effectiveness of their process control by completing 15 consecutive departmental verification tests with negative results before export of that product can commence. To demonstrate the establishment's ability to control the STEC hazard, one sample for STEC testing should be collected from each production date/shift, or more frequently as determined by the establishment's operational requirements.

Where a listed establishment pauses its exports of raw ground beef components for a period of time, the following options apply:

- Establishments not exporting grinding beef for a period of less than six months are not required to participate in monthly verification testing, nor are they required to complete 15 consecutive verification tests prior to resuming exports,
- Establishments not exporting grinding beef for six months or longer can either participate in monthly verification testing or can complete 15 consecutive verification tests prior to resuming exports.

The departmental verification program will be operated under the following conditions:

- Verification samples must be tested for all seven STEC serotypes
- The frequency of department verification testing (at least once per calendar month) will be determined by central office and will be based on the compliance history of the establishment
- The department on-plant officer will directly supervise the selection of the 12 test cartons and the collection of the test sample
- The department on-plant officer will take charge of the verification sample at the point of collection and will place the sample into a department tamper evident bag (NRS security satchel) and packed in department supplied NRS packaging material
- The sample will then be forwarded to a company nominated independent laboratory approved for department verification testing
- For a lot subjected to a verification test, the company can choose to use the result to support the health certificate endorsement that the lot tested negative (i.e. for a lot subjected to department verification testing with a negative result, an additional company test is not required)
- Where no USA or Canada eligible lots of raw ground beef components are available on a nominated day of verification testing, the establishment may request a different day for sampling when USA or Canada eligible product is available or they may request that like product, identified by the establishment, may be sampled on the original nominated date
- For the purpose of department verification testing the establishment may nominate a smaller than normal lot size
- All potential positive verification samples or potential positive verification follow-up samples must undergo confirmatory testing

- All *E. coli* O157 isolates from department verification samples must be forwarded to a department approved laboratory for the purposes of typing by pulsed field gel electrophoresis (PFGE), or other typing methodologies as required by the department equivalence agreement with the USA
- All transportation, storage and analysis costs will be covered by the establishment.
- If an establishment fails to complete one or more verification tests, the department will investigate the cause and depending on the finding, may implement corrective actions.

### 8.1.8. Reporting

All commercial and department verification test results must be reported immediately by the testing laboratory to the department on-plant officer (i.e. on-plant veterinarian) or departmental circuit inspector at the same time as they are advised to the establishment/establishment management. All positive commercial test results (potential or confirmed) and all department verification test results (positive and negative) must be reported immediately by the testing laboratory to the Food Safety Unit, Canberra ([FoodSafetyUnit@aff.gov.au](mailto:FoodSafetyUnit@aff.gov.au)), at the same time as they are advised to the establishment/establishment management and the on-plant officers.

All commercial test results, including potential and confirmed positive results, generated from samples collected at independent cold stores on behalf of packers or non-packer exporters, must be reported by the testing laboratory back to the originating packing establishment. These results must be reported to the respective department on-plant officer (i.e. on-plant veterinarian or food safety meat assessor) at the same time as they are advised to the exporter (packer or non-packer) and the cold store. All positive test results (whether potential or confirmed) must be reported immediately by the testing laboratory to the Food Safety Unit, Canberra at the same time as they are advised to exporter (packer or non-packer) and the cold store.

All test results (routine commercial tests, including test results received for samples collected at independent cold stores from products initially produced at their establishment, and departmental verification tests), and the disposition following a confirmed positive result, must be recorded by plant management, along with relevant sample information, in the STEC database in MEDC.

### 8.1.9. Actions on positive findings

#### 8.1.9.1. During commercial testing

All product in a lot destined for the USA or Canada from which a sample has been confirmed or deemed positive for one of the seven STEC must be retained and condemned or subjected to an approved treatment process validated to achieve at least a five log reduction in *E. coli* numbers in the product. Heat treatment of test

positive product should be equivalent to  $\geq 69.4^{\circ}\text{C}$  ( $157^{\circ}\text{F}$ ) for 10 seconds at the slowest heating point in the product being treated.

Some Australian state and territory governments have mandatory reporting requirements for the detection of STEC in meat. In these circumstances, the establishment should notify the on-plant officer who will provide an official State Regulatory Authority (SRA) Notification form. Completed notification forms are to be submitted to the department's Export Regulatory Integrity and Assurance Team by the on-plant officer, copying in the establishment and Food Safety Unit. The Export Regulatory Integrity and Assurance Team will advise the relevant SRA where appropriate by forwarding the notification form.

All testing, including positive results in commercial testing should be used by the establishment to inform the establishment of the effectiveness of their HACCP plan in relation to the control of STEC. The level of response expected of the company will increase where multiple detections are identified.

#### **8.1.9.2. During department verification testing**

In addition to complying with the requirements in 8.1.9.1, establishments upon being informed of a confirmed or deemed positive result in a department verification test must:

- Investigate their process and test records from the relevant production periods including livestock, slaughter, refrigeration and boning processes
- Reassess their current production process
- Review their HACCP plan in the parts related to the potential contamination of product with enteric pathogens
- Take any necessary corrective and preventive action and verify its effectiveness
- In the case of independent boning rooms, notify the supplying slaughter establishment from which product in the test positive lot was sourced
- Within 10 days of notification of the positive result, provide the department with a written report documenting the establishment's investigation, including supporting documents.
- Commence the collection of 15 follow-up STEC verification test samples. The department will supervise the collection and submission of these samples.

Follow-up STEC verification sample collection must start immediately after the positive verification test result is received with the aim of collecting the required 15 samples within a period of 30 working days. The samples should be collected from the same type of product which tested positive if available, and if not available the samples can be collected from beef manufacturing trimmings or from other raw ground beef components or bench trimmings. Establishment management in consultation with the OPV will nominate the lots from which the follow-up verification samples will be collected.

Establishments not producing to the US or Canadian Market at the time may sample from US or Canadian eligible product and may form smaller lots as small as 12 cartons and collect the follow-up verification samples from such lots, providing that all cartons are sampled to achieve the required N60 sampling.

Any confirmed positive result returned during follow-up verification testing will result in:

- Cessation of follow-up verification testing
- The establishment being required to conduct a new investigation into the detection of additional test positive product
- Upon completion of and reporting on the investigation by the establishment, the establishment will start a new round of 15 follow-up verification tests
- The department may consider sanctions<sup>3</sup> in the case of repeated failure in the verification testing program
- All verification and follow-up verification results will be recorded in MEDC.

## **8.2. STEC Testing of Raw Ground Beef Products for Export to Canada, the USA and its territories**

### **8.2.1. Purpose**

To advise export registered establishments and exporters of raw ground beef and ground beef patties to the USA and Canada of the requirement to:

- test those products for *E. coli* 0157 and to consider the testing of these products for other STEC of concern to the FSIS;
- participate in the departmental verification testing program whereby establishments exporting these products to the USA and Canada conduct one test for all seven STEC serotypes per month.

### **8.2.2. Scope**

This section covers the requirements for *E. coli* 0157 testing of raw ground beef products for export to Canada, the USA and its territories. Industry may wish to consider testing for other STEC. Industry must also participate in the departmental verification testing program.

Definitions in section [8.1.3](#) also apply in this section.

**Lot** - For the purposes of testing cartons of ground beef or ground beef patties for *E. coli* 0157 or STEC, it is the responsibility of the establishment to define a lot, taking into account processing criteria, cleaning schedules, the principle of 'same source

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<sup>3</sup> for more information on sanctions refer to the Critical Incident Response Guidelines.

materials' and other factors that may result in contamination of final product with pathogenic microorganisms. The establishment must have a supportable basis for defining the sampled lot. This basis should consider the possible connections between lots including lots produced from the same source material (source lots).

**Raw ground beef products** – are raw comminuted (chopped or ground) meat food products that are made from cattle (beef and/or veal), such as ground beef, hamburger, veal patties, and beef patty mix, that may be distributed to consumers as such. It is important to note that products comprised only of beef from advanced meat recovery systems are not considered a raw ground beef product.

Beef sausage products are not subject to the requirements for testing outlined in this manual.

### 8.2.3. Management Responsibilities

Exporters or establishment management exporting or intending to export raw, ground beef products to the USA and Canada should ensure that full details of their *E. coli* O157 (and STEC where applicable) testing program are approved as part of their HACCP plan and AA prior to commencing production.

Establishment management must also:

- Develop and implement a lot based sampling and testing program (work instructional material) based on the information in this manual and the requirements in Micor and ensure these are contained within the establishment AA
- Participate in the departmental verification testing program.
- Authorise testing laboratories to provide all relevant test results to the department on plant staff or circuit inspection staff at the same time as they are supplied to plant management or the exporter (refer Section 8.2.9)
- Ensure that all products can be traced and retained in the event of a positive result
- Ensure that where company staff are authorised request for permit (RFP) validators, that they do not validate an RFP unless:
  - the lot has is test negative for *E. coli* O157 (and where applicable other STEC)
  - processed product complies in full with the specific provisions of the AA
  - the daily record review (pre-shipment review) is completed and satisfactory; and
  - the product complies with the microbiological standards of this guideline.

### 8.2.4. Sourcing Raw Materials

As part of their purchase specifications establishments producing raw ground beef products must include a requirement for their suppliers to certify that each lot of raw

material (i.e. boneless beef used in the manufacture of raw ground beef products) received has been tested negative for at least *E. coli* 0157.

#### **8.2.5. Sampling, Labelling and Storage**

Establishments producing minced beef products for the USA and Canada must also conduct daily testing of their raw beef ingredients or of their raw ground beef products for *E. coli* 0157 and meet the *Salmonella* performance standard (refer Section 8.3.3.5).

When testing raw ground beef products, it is important that:

- Cartons sampled are selected at random to ensure the full range of raw ground beef products is available for sampling
- A minimum of five cartons must be sampled per lot
- Cartons should be selected and sampled at a point before they are lidded
- A minimum of 65 g is collected from each of the five cartons selected (total weight of no less than 325 g per lot)
- Sampling equipment is washed and sanitised between lots. Samples may be “grab sampled” or “core sampled”
- Samples must be labelled with adequate detail to allow identification and traceability to an individual lot (refer 8.1.4)
- Where testing is not going to commence immediately samples should be stored and/or transported according to the requirements in Section 8.1.6.3.

#### **8.2.6. Sample Dispatch**

Follow the procedure outlined in Section 6.3.

#### **8.2.7. Sampled Lot Retention**

This is a test and hold program, so the sampled lot must be maintained under the control of the establishment (i.e. able to be recalled) pending the results of the screening test or any confirmatory test.

In the event that a lot is confirmed as being positive or deemed positive for STEC, the lot must be retained and placed under department retention until a disposition is applied.

#### **8.2.8. Sample Analysis**

Samples must be sent to a department approved laboratory and analysed using department approved methods. The temperature of samples must be checked on arrival at the laboratory and must be  $\leq 7^{\circ}\text{C}$ .

All samples with a positive screening test must either undergo further testing for confirmation of *E. coli* O157/STEC in a departmentally approved confirmation laboratory or the result will be deemed to be positive.

Confirmatory testing must include isolation of colonies using an immunomagnetic separation technique followed by plating on selective, differential media (e.g. Rainbow Agar and/or CTSMAC).

All positive test results must be reported immediately to the department on-plant supervisor or inspectorial staff to ensure appropriate retention of product is applied

### **8.2.9. Department Verification Testing**

All establishments with USA and Canada listing for the production of export raw ground beef products must participate in the departmental verification testing program.

Where establishments with such a listing choose not to retain that listing or do not wish to participate in departmental verification testing, they:

- Must amend their AA to reflect this; and
- Must relinquish their listing for access to the USA and Canadian market for raw ground beef products.

Where an establishment has not previously exported raw ground products to the USA or Canada, that establishment must verify the effectiveness of their process control by completing 15 consecutive departmental verification tests with negative results before export of that product can commence. To demonstrate the establishment's ability to control the STEC hazard, one sample for STEC testing should be collected from each production date/shift, or more frequently as determined by the establishment's operational requirements.

Where a listed establishment pauses its exports of raw ground beef products for a period of time, the following options apply:

- Establishments not exporting raw ground beef products for a period of less than six months are not required to participate in monthly verification testing, nor are they required to complete 15 consecutive verification tests prior to resuming exports,
- Establishments not exporting raw ground beef products for six months or longer can either participate in monthly verification testing or can complete 15 consecutive verification tests prior to resuming exports.

Where a listed establishment produces both raw ground beef components and raw ground beef products in a single month, the verification sample may be drawn from either product and not necessarily both product types in that month.

The departmental verification program will be operated under the following conditions:

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- Verification samples must be tested for all seven STEC serotypes
- The frequency of department verification testing (at least once per calendar month) will be determined by central office and will be based on the compliance history of the establishment
- The department on-plant officer will directly supervise the selection of the five test cartons and the collection of the test sample
- The department on-plant officer will take charge of the verification sample at the point of collection and will place the sample into a department tamper evident bag (NRS security satchel) and packed in department supplied NRS packaging material
- The sample will then be forwarded to a company nominated independent laboratory approved for department verification testing
- For a lot subjected to a verification test, the company can choose to use the result to support the health certificate endorsement that the lot tested negative (i.e. for a lot subjected to department verification testing with a negative result, an additional company test is not required)
- Where no USA or Canada eligible lots of raw ground beef products are available on a nominated day of verification testing, the establishment may request a different day for sampling when USA or Canada eligible product is available or they may request that like product, identified by the establishment, may be sampled on the original nominated date
- For the purpose of department verification testing the establishment may nominate a smaller than normal lot size
- All potential positive verification samples or potential positive verification follow-up samples must undergo confirmatory testing
- All *E. coli* O157 isolates from department verification samples must be forwarded to a department approved laboratory for the purposes of typing by pulsed field gel electrophoresis (PFGE), or other typing methodologies as required by the department equivalence agreement with the USA
- All transportation, storage and analysis costs will be covered by the establishment.
- If an establishment fails to complete one or more verification tests, the department will investigate the cause and depending on the finding, may implement corrective actions.

#### 8.2.10. Reporting

All commercial and department verification test results must be reported immediately by the testing laboratory to the department on-plant officer (i.e. on-plant veterinarian) or departmental circuit inspector at the same time as they are advised to the establishment/establishment management. All positive commercial test results (potential or confirmed) and all department verification test results (positive and negative) must be reported immediately by the testing laboratory to the Food Safety Unit, Canberra ([FoodSafetyUnit@aff.gov.au](mailto:FoodSafetyUnit@aff.gov.au)), at the same time as they are advised to the establishment/establishment management and the on-plant officers.

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All commercial test results, including potential and confirmed positive results, generated from samples collected at independent cold stores on behalf of packers or non-packer exporters, must be reported by the testing laboratory back to the originating packing establishment. These results must be reported to the respective department on-plant officer (i.e. on-plant veterinarian or food safety meat assessor) at the same time as they are advised to the exporter (packer or non-packer) and the cold store. All positive test results (whether potential or confirmed) must be reported immediately by the testing laboratory to the Food Safety Unit, Canberra at the same time as they are advised to exporter (packer or non-packer) and the cold store.

All test results (routine commercial tests, including test results received for samples collected at independent cold stores from products initially produced at their establishment, and departmental verification tests), and the disposition following a confirmed positive result, must be recorded by plant management, along with relevant sample information, in the STEC database in MEDC.

## **8.2.11. Actions on positive findings**

### **8.2.11.1. During commercial testing**

All product in a lot destined for the USA or Canada from which a sample has been confirmed or deemed positive for one of the seven STEC must be retained and condemned or subjected to an approved treatment process validated to achieve at least a five log reduction in *E. coli* numbers in the product. Heat treatment of test positive product should be equivalent to  $\geq 69.4^{\circ}\text{C}$  ( $157^{\circ}\text{F}$ ) for 10 seconds at the slowest heating point in the product being treated.

Some Australian state and territory governments have mandatory reporting requirements for the detection of STEC in meat. In these circumstances, the establishment should notify the on-plant officer who will provide an official SRA Notification form. Completed notification forms are to be submitted to the department's Export Regulatory Integrity and Assurance Team by the on-plant officer, copying in the establishment and Food Safety Unit. The Export Regulatory Integrity and Assurance Team will advise the relevant SRA where appropriate by forwarding the notification form.

All testing, including positive results in commercial testing should be used by the establishment to inform the establishment of the effectiveness of their HACCP plan in relation to the control of STEC. The level of response expected of the company will increase where multiple detections are identified.

### **8.2.11.2. During department verification testing**

In addition to complying with the requirements in 8.2.10.1, establishments upon being informed of a confirmed or deemed positive result in a department verification test must:

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- Investigate their process and test records from the relevant production periods including livestock, slaughter, refrigeration and boning processes
- Reassess their current production process
- Review their HACCP plan in the parts related to the potential contamination of product with enteric pathogens
- Take any necessary corrective and preventive action and verify its effectiveness
- In the case of independent boning rooms, notify the supplying slaughter establishment from which product in the test positive lot was sourced
- Within 10 days of notification of the positive result, provide the department with a written report documenting the establishment's investigation, including supporting documents.
- Commence the collection of 15 follow-up STEC verification test samples. The department will supervise the collection and submission of these samples.

Follow-up STEC verification sample collection must start immediately after the positive verification test result is received with the aim of collecting the required 15 samples within a period of 30 working days. The samples should be collected from the same type of product which tested positive if available, and if not available the samples can be collected from beef manufacturing trimmings or from other raw ground beef components or bench trimmings. Establishment management in consultation with the OPV will nominate the lots from which the follow-up verification samples will be collected.

Establishments not producing to the US or Canadian Market at the time may sample from US or Canadian eligible product.

Any confirmed positive result returned during follow-up verification testing will result in:

- Cessation of follow-up verification testing
- The establishment being required to conduct a new investigation into the detection of additional test positive product
- Upon completion of and reporting on the investigation by the establishment, the establishment will start a new round of 15 follow-up verification tests
- The department may consider sanctions<sup>4</sup> in the case of repeated failure in the verification testing program
- All verification and follow-up verification results will be recorded in MEDC.

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<sup>4</sup> for more information on sanctions refer to the Critical Incident Response Guidelines.

## **8.3. *Salmonella* Testing for Ground Beef for Export to the USA**

### **8.3.1. Scope**

This section covers the requirement for *Salmonella* testing of ground beef for export to US or its territories

### **8.3.2. Requirements**

The FSIS requires that any ground beef exported to USA or its territories must be tested for *Salmonella* spp. Establishments intending to produce raw comminuted beef for export to the USA must include details of their *Salmonella* testing program in their AA. Establishments are required to undertake sampling and testing for *Salmonella* as outlined in this manual prior to making an application for an export permit (RFP).

### **8.3.3. Sample Collection and Testing**

#### **8.3.3.1. Sample Collection**

Raw ground product samples should be randomly collected:

- After the final grinding process
- Before any addition of spices or seasoning
- Prior to final packaging
- A minimum of 325 grams of ground beef should be aseptically collected and placed in a sterile plastic bag for shipment to a department approved laboratory for analysis
- All samples must be held refrigerated immediately after collection until time of analysis at the laboratory.

#### **8.3.3.2. Sample Shipment**

- Samples must be labelled with adequate detail to allow identification and traceability to an individual tested lot
- Where testing is not going to commence immediately samples should be stored and/or transported according to the requirements in Section 6.3.

#### **8.3.3.3. Sample analysis**

- Samples must be analysed for *Salmonella* spp. at a department approved laboratory using a department approved method.

#### **8.3.3.4. Reporting**

All *Salmonella* test results, potential (positive screening test), positive and/or negative, must be reported by the testing laboratory to the department on-plant

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supervisor or Central Office at the same time that they are reported to plant management.

#### 8.3.3.5. **Salmonella Performance Standard**

Table 9: Performance standard for *Salmonella* testing of ground beef for export to the USA

Performance standard (% positive for <i>Salmonella</i> in ground beef)	Number of samples tested (n)	Maximum number of allowable positives to achieve standard (c)
7.5	53	5

### 8.4. **Microbiological Testing requirements for Ready-To-Eat Meat Products eligible for export to the USA and its territories**

#### 8.4.1. **Purpose**

To advise export registered establishments of the microbiological testing requirements for ready-to-eat (RTE) meat and meat products exported to the USA and its territories.

#### 8.4.2. **Scope**

These requirements apply to all export registered establishments producing RTE meat products with  $\geq 3\%$  raw or  $\geq 2\%$  cooked meat or poultry for export to the USA and its territories.

#### 8.4.3. **Introduction**

The USA maintains a 'zero tolerance' policy for pathogens in RTE products and a positive result for *Salmonella*, *L. monocytogenes* or *E. coli* O157 will result in a lot being declared as adulterated with a disposition made of condemnation or a requirement for an approved decontamination step.

The FSIS advises that all establishments that produce RTE products that are exposed to the environment after lethality treatments will be required to develop written programs, such as Hazard Analysis and Critical Control Point (HACCP) systems, sanitation standard operating procedures (SOP) or other prerequisite programs, to control *L. monocytogenes* in the final product.

The following specific requirements apply to RTEs for export to the USA or its territories and should be read in conjunction with section 7.4.6 of this document.

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#### 8.4.4. Definitions

Refer to section 7.4.4

#### 8.4.5. Environmental Monitoring for *Listeria* spp.

All RTE export registered establishments must test their processing environment for the presence of *Listeria* spp. as per the requirements detailed in section 7.4.5. These requirements satisfy the *Listeria* environmental testing requirements of the USA. In the event of a positive detection proceed as detailed in section 7.4.5.

#### 8.4.6. Product Testing for *Listeria monocytogenes*

The following risk categories shall be applied to US eligible export products produced at the establishment **and exposed to the post-lethality environment**, to determine the minimum final product testing requirements. Non-USA product must be sampled at the minimum frequency specified in section 7.4.6, i.e. as for Alternative 1 products.

##### 8.4.6.1. Alternative 1

Alternative 1 - Product is treated with a post-lethality treatment that both reduces or eliminates *Listeria* on the product whilst also suppressing or limiting the growth of *L. monocytogenes*.

##### 8.4.6.2. Alternative 2A

Alternative 2A - Product is treated with a post-lethality treatment that achieves at least a one-log<sub>10</sub> (ten-fold) reduction in *L. monocytogenes*.

##### 8.4.6.3. Alternative 2B

Alternative 2B - Product is treated with an antimicrobial agent or process that allows no more than a 2-log<sub>10</sub> (one hundred-fold) increase in *L. monocytogenes* throughout the shelf life of the product – this alternative requires sufficient testing of food surfaces to show that environments are sanitary and free from *L. monocytogenes*.

##### 8.4.6.4. Alternative 3

Alternative 3 - Product is produced from an establishment where only sanitation measures are used to control product contamination.

#### 8.4.7. Sample and testing frequency

The Australian Regulatory Guidelines of the Meat Standards Committee require that RTE products must be tested for *L. monocytogenes* at least once every three months. This is a slightly higher frequency than the requirements of the USA where the

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minimum testing frequency of RTE products for *Salmonella* and *E. coli* O157 is once a quarter (Table 10).

Establishments exporting RTEs to the USA have the option to test each lot prior to export for *L. monocytogenes*, *Salmonella* and *E. coli* O157 (where applicable); or alternatively they can use a risk based testing frequency dependent on the highest risk classification (Alternative 1, 2 or 3) for each product for export to the USA (Table 10).

Table 10: Minimum testing frequency for pathogens in RTE product produced by the establishment

	No. of product tests per annum <sup>1</sup>			Surface tests (tests/line/year)
Product category	<i>L. monocytogenes</i> (25 g test portion)	<i>Salmonella</i> (325 g test portion)	<i>E. coli</i> O157 <sup>2</sup> (325 g test portion)	Food Contact Surfaces <sup>3</sup>
Commercially sterile	0	0	0	
No post lethality exposure but not commercially sterile	3	3	3	
Alternative 1	4	4	4	≥2
Alternative 2A	6	6	6	≥4
Alternative 2B	9	9	9	≥4
Alternative 3	15	15	15	≥12 (excluding deli meats and frankfurters)
Alternative 3 (deli meats and Frankfurters)	15	15	15	Large volume plant ≥48 <sup>4</sup> Small volume plant ≥24

<sup>1</sup> Includes non-risk-based testing requirements for all ready-to-eat meat products, excluding those that are commercially sterile

<sup>2</sup> Testing for *E. coli* O157 is only required for uncooked comminuted fermented meat products and cooked meat patties that contain meat of bovine origin exported to the US or its territories

<sup>3</sup> ≥30cm<sup>2</sup> for each surface if possible

<sup>4</sup> Annual production >600 tonnes

#### 8.4.7.1. Product sampling

Important things to consider in the collection of samples include:

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- A sufficient amount of intact packaged product should be collected to conduct the required tests as indicated by the product type in Table 10.
  - o 25±1 g for *L. monocytogenes*
  - o 325 ± 6.5 g for *Salmonella* and *E. coli* O157
- Samples must be traceable to the tested RTE batch produced for export
- Samples, unless already frozen when collected, must not be frozen during transport to the laboratory or allowed to directly contact frozen cooler blocks
- Sample temperature on arrival at the laboratory must be ≤ 5°C
- Analysis must commence no later than on day 2 after sample collection i.e. if the sample is collected on Monday then analysis must commence no later than on Wednesday of the same week
- All of the required tests can be performed on one sample.

#### 8.4.8. Actions upon receipt of Positive Results

RTE products that test positive for *L. monocytogenes*, *Salmonella* or *E. coli* O157 are adulterated and must be disposed of or otherwise treated as per procedures under the establishment's AA. Untreated product is not eligible for export to the USA or its territories.

**Note:** RTE products that test positive for *L. monocytogenes* but do not support its growth may be eligible for domestic markets or for export to markets that recognise a limit of <100 *L. monocytogenes* per g of product at the time of consumption.

RTE product that comes into contact with a surface that is contaminated with *L. monocytogenes* is also considered adulterated (except in the case of Alternative 1 and 2A where the post-lethality treatment effectively eliminates or reduces *L. monocytogenes*). If testing product contact surfaces for *Listeria* spp. or *Listeria* like organisms, positive results will trigger product hold and test for *L. monocytogenes* under the following conditions:

- Alternative 2B and 3 – after three consecutive positive results
- Alternative 3 (deli meats and frankfurters) – after two consecutive positive results. Under this scenario product testing for deli meats and frankfurters must be at a rate that provides a level of statistical confidence that the product is not adulterated.

If a sample is found positive for *Listeria* spp. and on further testing is found to be negative for *L. monocytogenes*, it is not adulterated. However, such a result is an indication that there is the potential presence of the pathogen and the controls in place may not be adequate. The establishment should take corrective action as indicated in its AA.

In the case of frankfurters or deli meats produced under Alternative 3, if a product or food contact surface tests positive for a pathogen, the establishment must verify the effectiveness of the corrective action by conducting follow-up environmental testing. If it is found that the establishment failed to implement effective corrective action

subsequent lots must be disposed of as for the positive lot, until satisfactory measures are implemented and verified.

#### 8.4.9. Establishment responsibilities

In addition to responsibilities in Section 6.2:

- Where they occur, post lethality treatments and antimicrobial agents must be documented in the establishment's HACCP program and AA
- Sanitation procedures must be maintained to minimise the risk of contamination in the post lethality environment
- For *L. monocytogenes*, any critical control point (CCP) identified in the HACCP plan must be validated by testing for *L. monocytogenes*
- If *Listeria* spp. controls are included in sanitation SOPs, their effectiveness must be verified through an establishment's microbiological surface monitoring program
- Testing of contact surfaces for verification of sanitation in the post-lethality environment may be for *L. monocytogenes*, *Listeria* spp. or *Listeria*-like organisms
- If a product contact surface tests positive for *Listeria* spp. or *Listeria*-like organisms, product must be tested for *L. monocytogenes*
- Provide advice to the senior department on-plant officer when samples are collected from product destined for the US (where department certification is required)
- Authorise testing laboratories to provide all relevant test results to the department on-plant staff simultaneously it is supplied to establishment management. Where the on-plant supervisor is not present at the establishment, a copy must be faxed/emailed to Mid – Operations in Canberra (Attention: Director – Food Safety, Animal Health and Welfare Unit)
- All ingredients (other than meat and vegetables) and additives must be received into the ingredient store and held pending examination and release by an authorised officer. The examination of the ingredients and additives shall include: a visual examination to ensure it is free from extraneous matter; and any tests specified in the AA. Where the examination shows that a batch of ingredients or additives is unsatisfactory, that batch shall be removed from the registered establishment
- Where establishment staff are authorised request for permit (RFP) validators they must not validate an RFP unless processed product complies in full with specific provisions of the USDA program approval, the daily record review (pre-shipment review) is completed and satisfactory and, the product complies with the microbiological specifications of this manual.

## 9. PARASITOLOGICAL TESTING

### 9.1. *Trichinella* in Meat

This section details the requirements for testing for *Trichinella* spp. in meat for export to the EU, Russia and any other market requiring *Trichinella* testing according to the EU Regulations.

#### 9.1.1. Scope

These requirements apply to all meat from domestic swine, equine, wild boar, crocodile produced for export. Meat from domestic swine that is frozen as detailed in “*Trichinella* Detection: Freezing Treatment for Domestically Produced Pork” is not covered by this section and is exempt from testing.

#### 9.1.2. Definitions

For the purposes of this section ‘*Trichinella*’ means any nematode belonging to species of the genus *Trichinella*.

**Note:** While *T. spiralis* is considered to be the most common species associated with human disease all species are considered infectious to humans.

#### 9.1.3. Background

The European Commission Regulation EC 2015/1375 requires testing of specified meats for *Trichinella* prior to or at post-mortem inspection. The department has approved the “magnetic stirrer method for pooled-sample digestion” for the analysis of samples for *Trichinella* spp.

Any alternate method must be approved by the department in writing before being implemented by a laboratory.

#### 9.1.4. Responsibilities

Establishment management must:

- Engage or provide a laboratory to perform *Trichinella* testing to support the certification of export meat
- Use a sampling and testing procedure that satisfies the requirements of sections 9.1.5 to 9.1.7 (inclusive) of this manual
- Ensure the procedure is captured in SOPs or work instructions based on the methods prescribed in this manual or its appendices
- Ensure the method used for the analysis of samples is exactly as detailed in Appendix 3 of this manual and must be approved by the department and carried out in a laboratory also approved by the department

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- Ensure that all products represented by the sample(s) being analysed are retained until a result has been obtained
- In the event of a positive result notify an officer of the department without delay.

The department or a department nominated certifying body will undertake a bi-annual assessment of the laboratory including sampling, testing, recording and reporting procedures.

### **9.1.5. Sampling**

Samples should be analysed on the day of collection. If testing cannot be performed on the day of collection, samples can be refrigerated but testing must commence within 24 hours of receipt.

#### **9.1.5.1. Domestic swine**

Unless the meat is frozen in accordance with Appendix 3 of this manual, the export of pork produced from domestic swine intended for the EU/Russian markets must be sampled at the abattoir prior to or at the post-mortem examination. A sample<sup>5</sup> must be collected from each carcase and the sample shall be examined for *Trichinella* in a department approved laboratory.

#### **9.1.5.2. Horses, wild boar and crocodile**

Any meat intended for export to the EU or Russia, which has been derived from the carcasses of horses, wild boar and crocodile, must be sampled at the abattoir or handling establishments prior to or at the post-mortem examination. A sample<sup>6</sup> (the weight of the sample is dependent on the species being tested) shall be collected from each carcase and the sample shall be examined in a laboratory approved by the department and identified in the establishment's AA.

Freezing of meat from horses, wild boar, crocodile and game meat does not make it exempt from testing as specified in this manual.

### **9.1.6. Traceability**

Pending the results of *Trichinella* testing the establishment must ensure full traceability of all carcasses and carcase parts is guaranteed. Carcasses may be cut up at a boning room attached to or separate from the abattoir provided that:

- The procedure is under the supervision of the department
- A carcase or the parts thereof will not have more than one boning room as its destination

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<sup>5</sup> The sample size and detail is defined in Appendix 3 of this manual.

<sup>6</sup> The sample size and detail is defined in Appendix 3 of this manual.

- The boning room is situated within Australia
- In case of a *Trichinella* positive result:
  - o the department will be notified immediately of the result; and
  - o all the parts of the carcase will be declared ineligible for human consumption (condemned).

This is a test and hold program meaning all carcasses, carcase parts or edible parts thereof which contain striated muscle tissue, may not leave the control of the establishment before the result of the *Trichinella* examination is available and shown to be negative.

### **9.1.7. Sample Testing**

#### **9.1.7.1. Competency**

Establishments must participate in any competency program specified by the department and meet all associated costs. All personnel involved in the examination of samples to detect trichinellae must be appropriately trained according to department's requirements and records of that training maintained.

Establishments must ensure that they implement a quality control program for the laboratory undertaking testing for *Trichinella* and that this is captured in the AA and included in annual audits of the AA.

For method of analysis refer to Appendix 3.

## **10. PRODUCT DISPOSITION**

### **10.1. Assessment of deterioration of refrigerated meat affected by refrigeration breakdown**

Guidance on making a disposition decision in the event of a departure from the required refrigeration parameters (i.e. time and temperature) is available in the department's *Guideline for the Application of the Refrigeration Index to Refrigeration of Meat and Meat Products* (<https://www.agriculture.gov.au/export/controlled-goods/meat/elmer-3/notices/2020/mn20-06>).

## Appendix 1. Technique for collecting swab samples

### a. Materials

- Container for carrying supplies
- Sterile gloves (optional with the alternate method)
- Sterile Template (use disposable templates, wire frame, stainless steel templates, and sterile disposable foil templates or equip with a supply of pre-sterilised templates)
- Whirl-pack™ type Collection method: Sterile specimen sponge in sterile Whirl-pack™ -type bag or equivalent;
- Diluents.

For *E. coli* and APC sampling use:

- 25 mL sterile Butterfield's Phosphate Diluent; or
- 25 mL of 0.1% Peptone Salt Solution<sup>7</sup> or Buffered Peptone Water.

For *Salmonella* sampling use 25 mL of Buffered Peptone Water<sup>8</sup>.

### b. Whirl-pack™ Method

A sampling sponge (which usually comes dehydrated and prepacked in a sterile bag) will be used to sample all the sampling sites on the carcass as follows:

- Ensure that all bags have been pre-labelled and all supplies are on hand, including the sampling template
- If a reusable template is used, it must be sterilised between each carcass (refer to the 'Procedure for sanitising reusable templates' in 7.1.4)
- Locate the sampling sites using relevant illustrations and directions in 7.1.4.
- While holding the sponge bag at the top corner by the wire closure, tear off the clear, perforated strip at the top of the bag
- Remove the cap from sterile diluents water bottle (diluents may differ depending on the target organism)
- Carefully pour about half the contents of the sterile diluent (approximately 10 mL) into the sponge bag to moisten the sponge. Recap the bottle
- Close the top of the bag by pressing the wire closure together. Use hand pressure from outside of the bag and carefully massage the sponge until it is fully hydrated (moistened). Sponges may be pre-moistened prior to entering the plant to sample the carcasses

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<sup>7</sup> Peptone Salt Solution - Dissolve 1g Peptone and 8.5 g sodium chloride in 1L of deionized water. Autoclave at 121 ±1°C for 15 min, pH after sterilization 6.9 ± 0.2, store in the dark at 0-5°C for one month

<sup>8</sup> Buffered Peptone Water: dissolve 10 g enzymatic digest of casein, 5 g NaCl, 9g Disodium hydrogen phosphate dodecahydrate and 1.5 g of Potassium dihydrogen phosphate in 1 L deionized water. Autoclave for 15 min at 121°C for 15 min. Final pH, 7.0 ± 0.2 at 25°C (as per AS 5013.10)

- Prior to collecting the sample, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening. DO NOT open the bag or touch the sponge with your fingers
- While holding the bag, gently squeeze any excess fluid from the sponge using hand pressure from the outside. The whole sponge should sit in the bag
- Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open. Set bag aside
- Put on a pair of sterile gloves (optional with the alternate method).
- Carefully remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand
- With your free hand, retrieve the template by the outer edge, taking care not to contaminate the inner edges of the sampling area of the template
- Locate the sample site e.g. flank for beef, horses and small stock; belly for pigs. Place the template over this location
- Hold the template in place with one gloved hand. Only the sponge should touch the sampling area. Take care not to contaminate this area with your hands
- With the other hand, wipe the sponge over the enclosed sampling area (10 cm × 10 cm or 5 cm × 5 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal direction. The pressure of swabbing should be as if you were trying to remove a stubborn stain from the carcass. The pressure should not be so hard as to crumble or destroy the sponge. The template may need to be “rolled” from side to side during swabbing since the surface of the carcass is not flat. This will ensure the 100 cm<sup>2</sup> or 25 cm<sup>2</sup> area is enclosed while swabbing
- Repeat these steps for the other sampling sites (brisket for beef, horses and small stock; ham for pigs) using the same side of the sponge used to swab the previous site
- Reverse the sponge and swab the third site as detailed above (butt for beef and horses; jowl for pigs; and mid-loin for small stock). For larger species, which would involve climbing the ladder or platform, ensure that you hold on to the rail with the hand used to hold the template. Once at a convenient and safe height for sampling, transfer template back to climbing hand (hand used to hold onto the rail while climbing the ladder or platform), and take care not to contaminate the inner edges of the template
- After swabbing all the sites, carefully place the sponge back in the sample bag. Avoid touching the sponge to the outside of the sample bag
- Uncap the previously used diluent bottle. Add the additional diluent (about 15 mL) to the sample bag to bring the total volume to approximately 25 mL (this step can be carried out back in the lab; taking care to use the corresponding sample bottle used to initially moisten the individual sponge)
- Expel excess air from the bag containing the sponge and fold down the top edge of the bag 3 to 4 times to close. Secure the bag by folding the attached wire tieback against the bag. Place closed sponge bag into the second bag and close the second bag securely

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- If samples are to be analysed at an on-site laboratory, begin sample preparation without delay. Ensure that intervals between collection and testing of samples are minimal
- If samples are to be analysed at an outside (offsite) laboratory, follow procedures detailed in Section 6.3. Transport of samples to NATA laboratory.

## Appendix 2. Calculation of *E. coli*, coliform and APC test results

### a. Swab Samples

Counts must be reported in CFU/cm<sup>2</sup>. Calculate the number of CFU/cm<sup>2</sup> area of meat surface as follows:

$$\text{CFU/cm}^2 = (\text{No. of colonies on plate}) \times \frac{(\text{Volume of diluent added})}{(\text{Area sampled})} \times (\text{Dilution Factor})$$

The dilution factor is used to adjust the results for dilutions undertaken during testing. Factors for 10 fold serial dilutions are as below:

Number of Tenfold Serial Dilutions	Dilution Factor
0	1
1	10
2	100
3	1000
4	10,000

The  $\frac{(\text{volume of diluent added})}{(\text{Area sampled})}$  is referred to as the sampling factor. Using cattle as an example. The initial sample is obtained by swabbing 300 cm<sup>2</sup> of carcass surface and suspending in 25 mL of diluent. The sampling factor is therefore  $\frac{25}{300}$  or 0.08. Sampling factors calculated for different species are provided below:

Species	Surface area sampled (in 25 mL diluent)	Sampling Factor
Cow/Bulls, Steers/Heifers	300 cm <sup>2</sup>	0.08
Horses/Mules/Donkey	300 cm <sup>2</sup>	0.08
Pigs	300 cm <sup>2</sup>	0.08
Calves	75 cm <sup>2</sup>	0.33
Sheep-lambs	75 cm <sup>2</sup>	0.33
Goats	75 cm <sup>2</sup>	0.33
Deer	75 cm <sup>2</sup>	0.33
Ratites	75 cm <sup>2</sup>	0.33
Dressed kangaroos	75 cm <sup>2</sup>	0.33
Pre-dressed kangaroos	25 cm <sup>2</sup>	1.00
Wild boars	50 cm <sup>2</sup>	0.50

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Examples of calculating CFU/cm<sup>2</sup> for large animals (300 cm<sup>2</sup> in 25ml) using combination of different dilutions and number of colonies is given below:

Number of colonies on plate	Number of Serial (10-fold) Dilutions	Dilution factor	Sampling Factor × Dilution Factor	Count CFU / cm <sup>2</sup>
0	0 (no dilution)	1	0.08	<0.08
1	0	1	0.08	0.08
100	0	1	0.08	8
10	1 (1 in 10)	10	0.8	8
100	1 (1 in 10)	10	0.8	80
100	2 (1 in 100)	100	8	800
100	3 (1 in 1,000)	1,000	80	8,000

Examples of calculating CFU/cm<sup>2</sup> for small animals (75 cm<sup>2</sup> in 25ml) using combination of different dilutions and number of colonies is given below:

Number of colonies on plate	Number of Serial (10-fold) Dilutions	Dilution factor	Sampling Factor × Dilution Factor	Count CFU / cm <sup>2</sup>
0	0 (no dilution)	1	0.33	<0.33
1	0	1	0.33	0.33
100	0	1	0.33	33
10	1 (1 in 10)	10	3.3	33
100	1 (1 in 10)	10	3.3	330
100	2 (1 in 100)	100	33	3300
100	3 (1 in 1,000)	1,000	330	33,000

## b. Surface Excision Samples

Surface excision samples must be reported in CFU/cm<sup>2</sup>. Excision samples are used to sample carcasses for certain species and in some instances for sampling surfaces of meat cuts i.e. primals, packed manufacturing meat.

The calculation of the number of CFU/cm<sup>2</sup> area of meat surface is as follows:

$$\text{CFU/cm}^2 = (\text{No. of colonies on plate}) \times \frac{(\text{Total volume of sample and diluent})}{(\text{Area sampled})} \times (\text{Dilution Factor})$$

The dilution factor is used to adjust the results for any further dilutions undertaken during testing i.e. if 1 mL of the initial sample is diluted with 9 mL of diluent prior to plating then the dilution factor is 10. The dilution factor is 1 for the initial dilution of the surface sample

Example: The undiluted sample is obtained by cutting a 25 cm<sup>2</sup> slice of surface tissue and homogenising in 9x the sample weight of diluent i.e. the sample was around 0.5 mm thick it would weigh approximately 12.5g and therefore 112.5 mL of diluent would need to be added (9 x 12.5g). If the undiluted sample is tested the number of bacteria on the plate must be multiplied by 125 (the total weight of sample and diluent; the sample is assumed to have a density of 1 i.e. 1g is equivalent to 1ml) and divided by 25 (the surface area sampled); this is equivalent to multiplying the count by 5 and gives the count in CFU per cm<sup>2</sup> of meat surface.

### c. Carton Meat samples

Results for testing of meat samples shall be reported as CFU per g of meat. The calculation of the number of CFU/g of meat is as follows:

$$\text{CFU/g} = (\text{No. of colonies on plate}) \times \frac{(\text{Total weight of sample and diluent})}{(\text{Sample weight})} \times (\text{Dilution Factor})$$

\* Total weight of sample and diluent = weight of sample + initial weight/volume of diluent

Example: 25 g of meat is homogenised in 225 ml (assumed to weigh 225g). When the undiluted sample is tested the number of bacteria on the plate must be multiplied by 250 (the total weight of sample and diluent) and divided by 25 (the original weight of the sample). This is equivalent to multiplying the count by 10 and gives the count in CFU per g of meat. As with previous examples the initial sample dilution factor is 1 and a 1:10 dilution of the initial sample has a dilution factor of 10, etc.

## Appendix 3. *Trichinella* Detection

### a. Magnetic Stirrer Method for Pooled Sample Digestion

#### Apparatus and reagents

- Knife or scissors and tweezers for cutting specimens
- A blender sufficiently powerful to finely blend samples
- Magnetic stirrer with thermostatically controlled heating plate or similar equipment capable of heating and stirring the sample during digestion
- Conical glass separation funnel, capacity of at least 2 litres

**Note:** To avoid loss of larvae through adherence to plastic surfaces glass apparatus must be used for all steps unless otherwise specified

- Sieve with mesh size 180 microns
- Funnel (glass or plastic), internal diameter sufficient to support the sieve while preventing leakage
- Glass beaker, capacity 3 litres
- Glass measuring cylinders, capacity 50 to 100 mL, or glass tubes
- Glass or plastic Petri dish (9 mm for use with a stereo-microscope), marked on their undersides into 10 × 10 mm squares
- Aluminium foil
- 25 % w/w hydrochloric acid (see note)
- Pepsin strength: 1: 10 000 NF (US National Formulary) corresponding to 1: 12 500 BP (British Pharmacopoeia) (see note)

**Note:** Different concentrations of acid and pepsin can be used as long as the final concentration in the digestion fluid is the same as specified in this method. Hydrochloric acid concentration must be calculated as weight of HCL per 100g of solution i.e. w/w

- Tap water heated to 46 to 48 °C
- A balance accurate to at least 0.1 g
- Pipettes (glass or plastic) of suitable volume
- A calibrated thermometer accurate to 0.5°C within the range 1 to 100°C.

#### Collecting of specimens and quantity to be digested

- In the case of carcasses of domestic swine, a specimen weighing at least 1 g is to be taken from a pillar of the diaphragm at the transition to the sinewy part
- In the case of domestic breeding sows and boars, a larger sample weighing at least 2 g is to be taken from a pillar of the diaphragm at the transition to the sinewy part
- In the absence of diaphragm pillars, a specimen of twice the size 2 g (or 4 g in the case of breeding sows and boars) is to be taken from the rib part or the breastbone part of the diaphragm, or from the jaw muscle, tongue or abdominal muscles

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- For frozen samples, a sample weighing at least 5 g of striated muscle tissue is to be taken for analysis
- For horse, wild boar and crocodile; specimens weighing at least 10 g are taken from the lingual or jaw muscle of horses; from the foreleg, tongue or diaphragm of wild boar; from the masseter, pterygoid and intercostal muscle of crocodile and 5 g used for analysis. For each digest the total weight of muscle examined must not exceed 100 g.
- The weight relates to a sample of meat that is free of all fat and fascia. Special attention must be paid when collecting muscle samples from the tongue in order to avoid contamination with the superficial layer of the tongue, which is indigestible and can prevent reading of the sediment.

## Procedure

### Complete pools (100 g of samples at a time)

- $16 \pm 0.5$  mL of 25% hydrochloric acid (or equivalent) is added to a 3 litre beaker containing 2.0 L of tap water, preheated to 46 to 48°C; a stirring rod is placed in the beaker, the beaker is placed on the preheated stirring hot plate or in a controlled temperature water bath/incubator and stirring started.

**Note:** Do not add water to acid as this may lead to boiling of the water and splashing of the HCl.

- $10 \pm 0.2$  g of 1:10000 pepsin (or equivalent i.e. 40g of 1:2500) is added to the HCL water mixture to make the digestion solution. Digest solution must be prepared fresh daily.

**Note:** Pepsin is added after the acid to avoid possible degradation by the concentrated acid prior to mixing.

- 100 g of pooled sample collected in accordance with the requirements detailed above is finely chopped in a blender for no more than 2 minutes. A small amount (i.e. 100 ml) of digestion fluid or tap water (taken from the 2L used to prepare the digestion solution) can be added to the blender to aid blending
- The blended meat is transferred to the 3 litre beaker containing the water, pepsin and hydrochloric acid. The blender is rinsed into the beaker with a small quantity of digestion fluid to remove any of the remaining meat
- The beaker is covered with aluminium foil and the temperature held at 44 to 46°C throughout digestion. The digestion temperature must be monitored and recorded
- During stirring, the digestion fluid must rotate at a sufficiently high speed to create a deep whirl without splashing
- Digestion continues for sufficient time (30 to 60 min) to ensure complete digestion of the sample. This will require some trial and error to determine the most suitable time for digestion of specific samples. At the completion of digestion the fluid is poured through the sieve into the sedimentation funnel.

**Note:** The digestion process is considered satisfactory if not more than 5 % of the starting sample weight remains on the sieve. The weight of residue must be verified and the results recorded.

- The digestion fluid is allowed to stand in the funnel for 30 minutes to allow sufficient time for the larvae to settle
- After 30 minutes, 40 mL of digestion fluid is quickly run off into the measuring cylinder or centrifuge tube. This 40 ml contains any larvae present in the sample.

**Note:** The digestion fluids and other liquid waste are to be retained until results are known.

- The 40 mL sample is allowed to stand for 10 minutes. After this time 30 mL of supernatant is carefully withdrawn by suction or pipette to remove the upper layers, leaving not more than 10 mL of sample in the cylinder.

**Note:** Care should be taken not to disturb any larvae settled at the bottom of the collection vessel. Liquid should be removed carefully from the top surface ensuring that the tip of the siphon or pipette remains just under the surface of the liquid. To avoid disturbing the sample the collection vessel should not be moved once the 10 min settling time has commenced.

- The remaining 10 mL sample containing any sediment is poured into a gridded Petri dish
- The collection vessel is rinsed with 5 mL (not more than 10 mL) of tap water, which is added to the sample in the Petri dish.

### **Microscopic Examination of Samples**

- Samples are examined under a stereo-microscope at 15-20x magnification
- Every square on the Petri dish must be systematically examined; this should take around 10 minutes per sample. To focus the microscope focus on the etched lines on the base of the Petri dish and then move the focal point up slightly (so that the lines are just out of focus and objects floating in the liquid are visible). This places any larvae in the focal point. To help with detection of larvae it is important to ensure that the volume of liquid placed in the Petri dish is as small as possible
- A department officer must be contacted in all cases of suspect or parasite-like shapes. To aid in identification, higher magnifications of 60 to 100 times must be available for use
- Digests are to be examined as soon as possible and no longer than 30 minutes from preparation.

### **Pools of more than 100 g (domestic pig only)**

- Where needed, up to an additional 15 g of samples (i.e. 3x5g) can be added to a pool of 100 g and examined together with these samples in accordance with the protocols outlined above
- Where more than 3x5g (15g) samples remain these must be examined as a complete pool

### **Pools of less than 100 g (all species)**

- For pools of up to 50 g (10 samples), the digestion fluid and ingredients may be reduced by half.

### Positive or indeterminate results

Where examination of a sample produces a positive or uncertain result, a further 20 g (50g for horse and wild boar) sample if available can be taken from each carcass pooled in the original sample to further isolate the positive carcass. Where meat from the implicated carcasses cannot be differentiated from other product all product produced on the day of the detection or uncertain result must be retained and disposed of in consultation with a department officer

- A sample of the parasite should be taken and stored in 90% ethanol and provided to the department Officer in charge. Suspect samples should be sent to AAHL for further classification
- For positive or suspect samples all fluids (digestive juice, supernatant fluid, washings, etc.) are to be decontaminated by heating to at least 60°C.

## b. Freezing Treatment for Domestically Produced Pork

### Freezing as a control option for *Trichinella* in Domestically Produced Pork

- Insulated packaging must be removed before freezing, except in the case of meat that is already at the required temperature throughout when it is brought into the refrigeration room or meat so packaged that the packaging will not prevent it from reaching the required temperature within the specified time
- The time when each consignment is brought into the freezer room must be recorded
- The temperature in the freezer must be at least -25°C
- Meat of a thickness of up to 25 cm must be frozen for at least 240 consecutive hours, and meat of a diameter or thickness of between 25 and 50 cm must be frozen for at least 480 consecutive hours. This freezing process must not be applied to meat that is thicker or of a larger diameter. The freezing time is calculated from the point when the temperature in the freezer reaches -25°C
- The following alternate time temperatures can be used:

Meat of a diameter or thickness of up to 15 cm must be frozen for one of the following time-temperature combinations:

- 20 days at -15°C
- 10 days at -23°C
- 6 days at -29°C

Meat of a diameter or thickness of between 15 cm and 50 cm must be frozen for one of the following time-temperature combinations:

- 30 days at -15°C
- 20 days at -25°C
- 12 days at -29°C

The following alternate time temperatures can be used for product of any thickness, where temperature is measured at the thermal centre of the product

- 106 hours at -18°C
- 82 hours at -21°C
- 63 hours at -23.5°C
- 48 hours at -26°C
- 35 hours at -29°C
- 22 hours at -32°C
- 8 hours at -35°C
- 1/2 hour at -37°C
- The temperature is to be measured using calibrated thermometers and recorded continuously. The thermometer probe is inserted in the centre of a cut of meat no smaller in size than the thickest piece of meat to be frozen
- This cut must be placed at the least favourable position in the freezer, not close to the cooling equipment or directly in the cold air flow
- The temperature charts must include the data numbers from the MTC/inventory/inspection record and the date and time of commencement and completion of freezing, and must be retained for one year after compilation.