Ministry for Primary Industries
Manatū Ahu Matua

Animal Products Notice

National Microbiological Database Programme

1 February 2023

Issued under the Animal Products Act 1999

New Zealand Government

TITLE

Animal Products Notice: National Microbiological Database Programme

COMMENCEMENT

This Animal Products Notice comes into force on 1 February 2023.

REVOCATION

This Animal Products Notice revokes and replaces the Animal Products Notice: National Microbiological Database Programme issued on 2 August 2022.

ISSUING AUTHORITY

This.Animal Products Notice is issued under section 167(2) of the Animal Products Act 1999 and supplements the Animal Products Regulations 2021.

Dated at Wellington, 30 January 2023

[signed]

Allan Kinsella
Director Assurance
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(acting under delegated authority of the Director-General)

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Introduction

This introduction is not part of the Animal Products Notice, but is intended to indicate its general effect.

Purpose

This notice supplements the requirements of the Animal Product Regulations 2021 and sets requirements for microbiological sampling and testing of animal material and animal products intended for human consumption.

Background

The National Microbiological Database (NMD) Programme is a standardised microbiological sampling and testing programme to provide ongoing monitoring of microbiological process control across all industry participants.

Who should read this Animal Products Notice?

You should read this notice if you are:

- an operator who processes red meat or poultry intended for human consumption; or
- a recognised laboratory that tests red meat or poultry intended for human consumption

Why is this important?

A failure to comply with this notice may be an offence under section 135(1)(c) of the Animal Products Act 1999 and may result in further action by an animal products officer.

Document History

Version Date	Section Changed	Change(s) Description
1 July 2022	-	New Notice
2 August 2022	4.13 *Campylobacter* Testing	Changes to *Campylobacter* confirmation test method
1 February 2023	1.5 Transitional provision relating to sample-takers	Clarified transitional provision references

Part 1: Preliminary provisions

1.1 Application

(1) This notice applies to the sampling and testing of red meat and poultry intended for human consumption.

1.2 Interpretation

(1) In this notice, unless the context otherwise requires:

APC means aerobic plate count

CFU means colony forming units

CPT means the *Campylobacter* performance target for meat chickens described in clause 4.17

ESR means the Institute of Environmental Science and Research

HACCP means Hazard Analysis and Critical Control Point

KTP means a key technical person engaged by a laboratory as a qualified expert in an NMD test (see clause 2.14)

laboratory means a recognised laboratory that has one or more NMD tests in its scope of recognition; and in relation to an operator, means the laboratory engaged by the operator to conduct NMD tests for the purpose of this Notice

missed sample means a failure by the operator to ensure samples are collected as required under this notice

moving window means a set of samples taken over a set period of time in which the addition of the latest set of samples to the window displaces the oldest dated set of samples

NMD database means the database established and maintained by the Director-General for the purposes of this Notice

NMD test means any of the following tests:

a) in relation to red meat:
    i) APC Petrifilm™ as described in clause 3.21.6;
    ii) APC spiral plater as described in clause 3.21.7;
    iii) APC spread plate as described in clause 3.21.5;
    iv) *Escherichia coli* Petrifilm™ as described in clause 3.22;
    v) *Salmonella* as described in clause 3.23; and

b) in relation to poultry:
    i) *Campylobacter* as described in clause 4.13;
    ii) *Salmonella* as described in clause 4.14.

NZRM means the New Zealand Reference Culture Collection, Medical Section, Catalogue of Strains

operator means the operator of an animal products business that carries out:

a) the primary processing of red meat or poultry animal material for human consumption; or
b) the secondary processing, by cutting and boning, of meat animal product for human consumption that is derived from bovine, bobby calf, caprine, or cervine animals

poultry means animal material or animal product derived from any of the following:

a) a chicken (being a bird of the species *Gallus gallus);*

b) a duck of the species *Anas platyrhynchos* domestica, *Anas pekin* (Peking ducks), *Cairina moschata* (Muscovy ducks), or a hybrid of these (known as mulard or moulard ducks);
c) a turkey (being a bird of the species *Meleagris gallopavo*.

premises includes all areas within the boundaries of an operator's RMP

processing week means:

a) in relation to red meat:

    i) a week starting on a Monday during which red meat is processed; and

b) in relation to poultry:

    i) for standard throughput poultry, a block of 5 consecutive processing days, not overlapping with the previous or next block of 5 processing days;
    ii) for VLT poultry, a week, starting on a Monday, during which VLT poultry is processed.

product type means a:

a) carcass; or
b) primal cut; or
c) bulk meat product.

red meat means:

a) in relation to primary processing, animal material (other than deer velvet) derived from any of following species, whether farmed or hunted, and intended for human consumption:

    i) bovine (cattle, buffalo);
    ii) bobby calf (treated as a separate species for the purpose of this notice);
    iii) caprine (goats);
    iv) cervine (deer);
    v) ovine (sheep);
    vi) porcine (pigs);
    vii) ratite (ostriches and emus).

b) in relation to secondary processing by cutting and boning, meat intended for human consumption and derived from any the following species:

    i) bovine;
    ii) bobby calf;
    iii) caprine;
    iv) cervine.

sample-taker means a person who is either a Level 1 sampler or a Level 2 sampler

season means:

a) for bobby calves, a calendar year; and
b) for all other species, the period beginning the first Monday in October and ending the Sunday before the first Monday in October of the following year

set of samples means:

a) in relation to meat:

    i) for standard throughput meat, the samples taken over one processing week; or
    ii) for VLT red meat, the samples taken over one month; and

b) in relation to poultry:

    i) for standard throughput poultry, the samples taken over one processing week; or
    ii) for VLT poultry, 3 samples taken from one processing day in a processing week.

SPS means the *Salmonella* performance standard, which is:

a) for red meat, the performance standard for bovine, caprine, ratite, and bobby calf described in clause 3.28; and
b) for meat chickens, the performance standard described in clause 4.16

standard throughput premises means:

a) in relation to red meat, premises at which, at the beginning of a season, it can reasonably be expected:
    i) in relation to bobby calf, bovine, caprine, cervine, ovine, or porcine species an operator will process 10,000 or more animals of that species in the season; or
    ii) in relation to ratites, premises at which an operator processes one or more animals of that species in the season.
b) in relation to poultry, premises at which, at the beginning of a season, it can reasonably be expected that more than 1,000,000 birds will be processed in the season.

technical failure means a failure to produce a test result following sampling as a result of:

a) samples not being delivered to the laboratory in the required time, or within the correct temperature range; or
b) a laboratory error

TNTC means too numerous to count

verifier, in relation to an operator, means the verifier or verifying agency of the operator's RMP

VLT means very low throughput, and:

a) in relation to red meat, means the operator is reasonably expected to process:
    i) in relation to bovine, caprine, cervine, ovine or porcine species fewer than 10,000 animals of that species in the season; and
    ii) in relation to bobby calf, fewer than 10,000 animals in the calendar year; and
b) in relation to poultry, fewer than 1,000,000 birds will be processed in the season.

zero dilution means the initial suspension of the bulk meat or poultry carcass rinsate sample.

(2) Terms used only in Part 3 are defined in clause 3.2, and terms used only in Part 4 are defined in clause 4.2.

(3) Any term defined in the Act, the Regulations, or the Animal Products Notice: Production, Supply and Processing and used in this Notice but not defined has the meaning given in the Act, Regulations or Animal Products Notice: Production, Supply and Processing, unless otherwise specified.

1.3 Records to be kept for 4 years

(1) If an operator or a laboratory is required by this Notice to keep specific records, the operator or laboratory must ensure that the records are legible and are stored:

a) for a minimum of 4 years; and
b) in a manner that:
    i) protects them from damage; and
    ii) is an easily accessible form.

1.4 Material incorporated by reference

(1) Under section 168 of the Act, the current edition of the Meat Industry Microbiological Methods (MIMM) is incorporated by reference into this notice as a standard work of reference.

1.5 Transitional provision relating to sample-takers

(1) A person who was an approved sampler for the purpose of the Animal Products Notice: Specifications for National Microbiological Database Programme issued 21 April 2021 is deemed to be a Level 1 sampler from 1 July 2022.

(2) A person who was a certified trainer for the purpose of the Animal Products Notice: Specifications for National Microbiological Database Programme issued 21 April 2021 is deemed to be a Level 2 sampler from 1 July 2022.

Part 2: Requirements applying to both red meat and poultry

2.1 Application

(1) This Part applies to:

a) an operator who processes red meat or poultry for human consumption; and
b) a laboratory that tests red meat or poultry for the purposes of this Notice.

2.2 Demographics form to be provided by operators

(1) Before processing any species of red meat or poultry, an operator must submit to the Director-General a completed demographics form for each species intended to be processed.

(2) The demographics form must include all the following:

a) the name, RMP identifier, physical and postal addresses of the operator's premises;
b) the name of operator and contact details (phone, cell phone and email);
c) the name of operator's NMD controller and contact details (phone, cell phone and email);
d) the name and identification number of the laboratory conducting NMD testing and coordinating the NMD sampling programme on behalf of the operator;
e) whether the operation is a standard throughput or VLT premises;
f) procedural details of the processing operation;
g) for poultry only, the location and reference number for each farm and reference number for each poultry shed.

(3) If any of the details on the demographic form change, the operator must notify the Director-General, in writing, within 1 week from the date the change occurred.

Guidance

- Demographic forms are available at: NMD Documents
- Submit completed forms and notifications to the Director-General: nmd@mpi.govt.nz

2.3 Operators to appoint NMD controller

(1) Every operator must appoint an NMD controller for the purpose of this Notice.

(2) The NMD controller's role is:

a) to be the point of contact, for the purpose of this Notice, with the Director-General and with the laboratory; and
b) to oversee day-to-day compliance at the operator's processing premises with the requirements of this Notice (such as those relating to the taking, storing, packing, and transporting of samples, and record-keeping).

(3) The operator may be the NMD controller.

(4) The operator must not appoint as an NMD controller any person who works for the laboratory.

(5) The operator must notify the Director-General and the laboratory, in writing, of:

a) the name and contact details of an NMD controller, within 1 week after the NMD controller is appointed; and
b) any changes to the appointment within, 1 week from the date the change occurred.

2.4 Operators to engage sample-takers

(1) Every operator must engage at least one sample-taker to take samples for the purpose of this Notice.

(2) A sample-taker engaged to take samples must be an employee of either an operator or laboratory.

(3) Within 1 week of engaging a sample-taker for the purpose of this Notice, the operator must notify the laboratory, in writing, of the following:

a) the name of the person;
b) whether the person is a Level 1 sampler or a Level 2 sampler;
c) the species and product types for which the person is trained to take samples;
d) the date of the person's training;
e) the name of the Level 2 sampler who trained the person.

(4) If the person is a Level 2 sampler, the operator must also send the laboratory evidence that the person has successfully completed the course required by clause 2.15.

(5) The operator must notify the laboratory of any change to the engagement, and any change in the details notified to the laboratory, within 1 week after the change.

2.4.1 Ensuring sample-taker competency

(1) An operator must ensure that a sample-taker engaged by the operator takes the samples required by this Notice in accordance with this Notice.

(2) The operator must ensure that the competency of samples-takers is maintained, and for that purpose must arrange for a review of each sample-taker, at least once every 12 months, by a Level 2 sampler who is engaged by the laboratory.

2.5 Testing of samples

(1) Every operator must engage the services of a laboratory to conduct NMD tests for the purpose of this Notice.

(2) The operator must provide equipment to refrigerate and store samples, prior to transport to the laboratory.

(3) The operator must inform the laboratory, in writing, of production schedules and plant closures that could affect NMD sampling.

2.6 Operators providing samples to laboratory

(1) The operator must provide every sample to the laboratory within the time agreed between the operator and the laboratory to ensure the laboratory can initiate analysis within 30 hours from the time the first sample was collected (as required by clauses 3.18 and 4.10).

(2) The operator must ensure that every sample provided to a laboratory:

a) is the appropriate sample for the relevant NMD test to be performed; and
b) has not deteriorated in any material respect since it was collected.

(3) If a problem occurs during the taking, storing, packing, or transport of a sample that means that a test result cannot be obtained from the sample, the operator must make every effort to re-sample in the same processing week or same processing day (standard throughput poultry premises).

(4) If, as a result of a non-compliance during the taking, storing, packing or transport of a sample, a sample does not meet the requirements of this Notice, the operator must report the details of the non-compliance to the Director-General, in writing, within 1 week of the non-compliance.

(5) The operator must take corrective actions to address any failure to meet the sampling requirements in this notice.

2.7 Operator to review test results

(1) Every operator must review the NMD test results received from a laboratory on samples taken under Part 3 and Part 4:

a) within 7 days (or up to 14 days in the case of a *Salmonella* detection) from the date the samples were received by the laboratory; and
b) in a manner that facilitates analysis of trends and early identification of loss of process control.

(2) The operator's review must:

a) check that the results accurately represent processing done; and
b) respond to any breach of the National Microbiological Criteria, the SPS, or the CPT, as relevant.

Guidance

- If an operator enters authorised NMD results into a database other than the NMD database for analysis of data, trends etc., alterations may be made to the format or presentation of the results provided that such alterations do not affect the results themselves in any substantive way.

2.8 Laboratory to appoint NMD laboratory manager

(1) Every laboratory must appoint an NMD laboratory manager to be the point of contact in relation to this notice for the Director-General and operators who engage the laboratory to do NMD tests.

(2) The laboratory must notify the Director-General and each operator who engages the laboratory to do NMD tests, in writing, of:

a) the name and contact details of the NMD laboratory manager; and
b) any changes to the appointment or contact details of the NMD laboratory manager.

(3) Notification under subclause (2) must be given within 1 week after the relevant appointment or change to the appointment or details of the NMD laboratory manager.

Guidance

- Submit notifications to the Director-General to: nmd@mpi.govt.nz

2.9 Laboratory to engage Level 2 sampler

(1) Every laboratory that undertakes NMD tests must engage at least one Level 2 sampler who is an employee of the laboratory.

(2) Within 1 week of engaging a Level 2 sampler, the laboratory must notify the Director-General, in writing, of the following:

a) the name of the person;
b) the species and product types for which the person is trained to take samples;
c) the date of the person's training and evidence of the successful completion of the course required by clause 2.15;
d) the name of the Level 2 sampler who trained the person.

(3) The laboratory must notify the Director-General of any change to the employment, and any change in the details notified to the Director-General, within 1 week after the change.

2.10 Laboratory to keep records of sample-takers

(1) Every laboratory must keep records of:

a) the details relating to sample-takers engaged by operators, as provided to the laboratory under clause 2.4(2); and
b) the details referred to in clause 2.9 of every sample-taker engaged by the laboratory.

(2) The laboratory must provide to the Director-General, and update as necessary:

a) the details referred to in subclause (1)(a), as soon as practicably after the laboratory receives them from an operator; and
b) the details referred to in subclause (1)(b), as soon as practicable after the laboratory engages a sample-taker.

2.11 Receipt of samples by laboratory

(1) A laboratory must not accept a sample for an NMD test unless the sample:

a) has been collected by a sample-taker whose details are held by the laboratory; and
b) is in a condition that is suitable, as specified in Part 3 or Part 4, for the requested NMD test to be performed.

(2) If a laboratory does not accept a sample for an NMD test because the sample does not meet the requirements in subclause (1), the laboratory must notify the operator, in writing, within 24 hours from the time the sample was received.

(3) A laboratory must enter sample description information (see clauses 3.16 and 4.9) in the NMD database if they are not entered by the sample-taker.

(4) If a laboratory receives samples with a carton seal or intervention seal, the laboratory must record:

a) the number of the seal if individually numbered; and
b) whether the seal was intact or not.

(5) If a carton seal or intervention seal is not intact on arrival at a laboratory, the laboratory must treat the samples as unsuitable for testing and notify the Director-General and the operator of the situation.

2.12 Temperature requirements for samples

(1) At least every 6 months the laboratory must check, using a temperature recorder, that the requirements in Part 3 and Part 4 relating to the temperature of samples between their dispatch from an operator for testing and testing (see clauses 3.17 and 4.10) are being complied with.

(2) If a temperature review identifies any non-compliance with the temperature requirements, the laboratory must notify the relevant operator, and the Director-General, in writing, within 1 week after the date of the review.

2.13 Laboratory testing and results

(1) A laboratory must ensure that all NMD tests are conducted, and results are calculated, in accordance with the requirements in Parts 3 or 4, as relevant.

(2) The laboratory must ensure the results:

a) identify the premises from which the samples were taken, the process descriptors, and the sample description information (see clauses 3.16 and 4.9); and
b) are submitted to the operator and entered into the NMD database:

i) within 24 hours of completion of testing, but no later than 1 week after receipt of samples; or
ii) in the case of a test for detection of *Salmonella*, no later than 2 weeks after receipt of samples.

(3) Within 24 hours of completing a test, the laboratory must notify the operator of any presumptive results that indicate a breach of the National Microbiological Criteria, the SPS, or the CPT has occurred.

(4) The laboratory must ensure that, if a problem occurs during testing that results in a sample not meeting testing requirements, the problem is reported to the Director-General, in writing, within 1 week after receipt of sample.

2.14 Laboratory to appoint KTP

(1) A laboratory must appoint a KTP for each NMD test conducted within the laboratory's scope of approval.

(2) The laboratory must ensure that the KTP for an NMD test:

a) reconciles the results calculated under Parts 3 or 4 with the original colony counts or presence/absence results recorded at the conclusion of the test; and
b) signs the laboratory report that has the test results that are entered into the NMD database.

2.15 Sample-taker competencies

2.15.1 Level 1 samplers

(1) A person is competent to be a Level 1 sampler in relation to specified species and product types only if they have been trained in sample-taking by a Level 2 sampler who is competent to take samples for that species and product type.

2.15.2 Level 2 samplers

(1) A person is competent to be a Level 2 sampler in relation to specified species and product types only if the person:

a) has successfully completed a course provided by or on behalf of the Director-General for assessing the competency of samplers of those species and product types; and
b) has at least 1 season of experience in taking samples under Part 3 or Part 4 in relation to those species and product types.

(2) Any course provided by or on behalf of the Director-General to assess competency of a person to be a Level 2 sampler must include all the following matters for any species and product type of red meat or poultry:

a) the criteria for sample collection;
b) sample packaging; and
c) sample transport.

2.16 Sample-taker details

(1) The Director-General must maintain records, on the MPI website, of the following details provided to the Director-General by a laboratory under clause 2.10:

a) the sample-taker's name;
b) the species and product types for which they are competent to take samples;
c) whether the person is a Level 1 sampler or a Level 2 sampler;

d) for a sample-taker employed or engaged by an operator, the name of the operator and the operator's laboratory; and
e) for a sample-taker employed by a laboratory, the name of the laboratory.

Guidance

- The sample-taker details are: NMD Level 1 Samplers and NMD Level 2 Samplers.
- A laboratory may contact the Director-General at nmd@mpi.govt.nz to provide details of Level 1 samplers and Level 2 samplers.

Part 3: NMD Red Meat Programme

3.1 Application

(1) This Part applies to:

a) an operator required to take samples for the purpose of NMD testing of red meat; and
b) a laboratory engaged by an operator to undertake the required NMD tests.

3.2 Interpretation

(1) Additional terms in this Part, unless the context otherwise requires:

bobby calf means an unweaned bovine calf that is less than 45 kilograms in weight as a dressed carcass

bulk meat means a piece of red meat that is packed by the operator into a carton in which there are 2 or more pieces of meat that are not individually wrapped

dsBPW means double strength buffered peptone water

initial item means the first carcass, primal cut, or carton of bulk meat to be sampled

primal cut means a main muscle from a red meat carcass

PSW, in relation to *Salmonella* sampling, means a primary sampling window (set out in clause 3.5)

run means a period of processing between planned process breaks

ssBPW means single strength buffered peptone water

SW, in relation to *Salmonella* sampling, means sampling window (set out in clause 3.5)

Visual lean means the observed fat content of the red meat, and, for purposes of the NMD, CL (chemical lean) means the same as visual lean.

3.3 NMD samples and tests required

(1) Operators must ensure that the tests required in Table 1 are completed for each product type to be sampled for each species listed, except that operators who process product not requiring official assurances need only sample carcasses.

Table 1

Species	Product type to be sampled	NMD required tests
Bovine Caprine	Carcass, primal cut, bulk meat, and post chill carcass	APC and generic *E. coli* A *Salmonella* composite of each product type (except post chill carcass) sampled during a PSW, or SW
Cervine	Carcass and primal cut	APC and generic *E. coli*
Porcine	Carcass	APC and generic *E. coli*
Bobby calf	Carcass, primal cut, bulk meat and post chill carcass	APC and generic *E. coli* A *Salmonella* composite of each product type (except post chill carcass) sampled during a SW
Ovine	Carcass	APC
Ratite	Carcass	APC and generic *E. coli* A *Salmonella* composite of carcasses sampled each week for PSW, or SW

3.4 Number and frequency of samples required

3.4.1 Standard throughput premises

(1) Operators of standard throughput premises must ensure that the number of each product type is taken for sampling at the stated frequency for each species listed in Table 2, except that operators who process product not requiring official assurances need only sample carcasses.

Table 2: Standard Throughput Premises

Species	Product type	Number of product type to be sampled and frequency of sample taking
Bobby calf, bovine, caprine, ovine, porcine, ratite	Carcass	5 on 1 processing day in each processing week
Cervine	Carcass	3 on 1 processing day in each processing week
Bobby calf, bovine, caprine	Primal cuts	5 on 1 processing day in each processing week
Cervine	Primal cuts	2 on 1 processing day in each processing week
Bobby calf, bovine, caprine	Bulk meat product	5 on 1 processing day in each processing week
Bobby calf, bovine, caprine	Post chill carcass (US listed premises only)	5 per week for the first 6 processing weeks of each season

3.4.2 VLT premises

(1) Operators of VLT premises must ensure that the number of each product type is taken for sampling at the stated frequency for each species listed in Table 3, except that operators who process product not requiring official assurances need only sample carcasses.

Table 3: VLT Premises

Species	Product type	Number of product type to be sampled and frequency of sample taking
Bobby calf, bovine, caprine, ovine, porcine, ratite	Carcass	5 on 1 processing day in each calendar month during which processing occurs
Cervine	Carcass	3 on 1 processing day in each calendar month during which processing occurs
Bobby calf, bovine, caprine	Primal cuts	5 on 1 processing day in each calendar month during which processing occurs
Cervine	Primal cuts	2 on 1 processing day in each calendar month during which processing occurs
Bobby calf, bovine, caprine	Bulk meat product	5 on 1 processing day in each calendar month during which processing occurs
Bobby calf, bovine, caprine	Post chill carcass (US listed premises only)	5 per week for the first 6 processing weeks of each season

3.5 Primary sampling window (PSW) and sampling window (SW) requirements for *Salmonella*

3.5.1 PSW for bovine, caprine, ratite

(1) Operators who process bovine, caprine, or ratite species must undertake *Salmonella* sampling in accordance with clause 3.3 when first commencing slaughter and dressing of each species under a newly registered RMP or significant amendment to that RMP and continue until there are 16 consecutive clear composite samples for each product type (PSW).

Guidance

- This would apply when an RMP is first registered for an operator of new premises or when a significant amendment to an existing RMP is made to include slaughter and dressing of an additional species to which *Salmonella* sampling applies.

3.5.2 SW for bovine, caprine, and ratite

(1) After meeting clause 3.5.1 (1), every standard throughput premises operator who processes bovine, caprine, or ratite must conduct *Salmonella* sampling for each product type for each season until there are 6 consecutive clear composite samples (SW) for that season.

Guidance

- If the PSW is completed over more than 1 season (e.g. the premises begins operating near the end of a season), a minimum of 6 clear weeks of sampling must be completed in the second season to meet the SW requirement. This may mean sampling for longer than the initial 16 weeks required for the PSW.

3.5.3 SW for bobby calves

(1) In each year every operator who processes bobby calves must conduct *Salmonella* sampling until there are 6 consecutive clear composite samples for each product type.

3.5.4 Restarting PSW or SW

(1) For the purpose of *Salmonella* sampling, the PSW or SW must be restarted at week 1 if there is a missed sample, but not if there is a technical failure.

3.6 Taking samples

3.6.1 Carcasses

(1) Operators must ensure that for carcass samples:

a) samples are taken no later than 30 minutes after post-mortem examination of carcasses;
b) carcasses are sampled before any physical procedure or chemical intervention that may impact on the NMD sampling sites is undertaken;
c) samples are not taken from carcasses detained by post-mortem examiners; and
d) carcass description information as per clause 3.16 is recorded at time of sampling directly into the NMD database or on the sample submission form.

Guidance

- Procedures that may impact on sampling sites include ones that redistribute contaminants (such as carcass contact), add contaminants (such as handling by workers), or remove contaminants (such as carcass washing or gross hot trimming).

3.6.2 Primal cuts

(1) Operators must ensure that for primal cut samples:

a) only fresh boned primal cut product is sampled;
b) samples are taken from a fell (outside) surface of the primal cuts;
c) the primal cut selected corresponds to the equivalent hindquarter NMD carcass sampling site as per clause 3.13 for the species concerned, with the exception of cervine primal cuts where both hindquarter and forequarter primal cuts can be selected;
d) samples are taken immediately prior to vacuum packaging, wrapping, or packing into cartons;
e) primal cut description information as per clause 3.16 is recorded at time of sampling directly into the NMD database or on the sample submission form; and
f) for cervine primal cuts:

i) a sample-taker picks the sample that fits the randomly selected time and records the actual cut sampled;
ii) intact cuts are sampled as the preferred choice;
iii) if at the time of sampling, all cuts are being silver-skinned (de-sinewed), then samples are taken immediately prior to packing; and
iv) a sample-taker records whether the cuts are silver-skinned or intact.

3.6.3 Bulk meat product

(1) Operators must ensure that for bulk meat product samples:

a) only fresh boned bulk meat product is sampled;
b) if premises produce product in the 85-95 visual lean range, samples are taken from product in this range;
c) if premises do not produce product in the 85-95 visual lean range, samples are taken from product in the 65-85 visual lean range;

d) samples are taken immediately prior to closing and strapping the carton before chilling or freezing; and
e) bulk meat product description information as per clause 3.16 is recorded at time of sampling directly into the NMD database or on the sample submission form.

3.6.4 Post chill carcasses (for US listed premises only)

(1) Post chill carcass sampling applies only to operators conducting cold and warm boning processes for bovine, bobby calf, and caprine species, whether operating standard throughput premises or VLT premises.

(2) Operators must ensure that for post chill carcass samples:

a) sampling is commenced on starting the NMD red meat programme and then is undertaken annually at the start of the season;
b) sampling is rotated on a weekly basis around the chillers, to a maximum of 6 chillers (5 samples per chiller) during each annual period;
c) sampling is continued until 30 carcasses (at 5 carcasses per week for 6 consecutive processing weeks) have been monitored. If sampling takes more than 6 weeks it must be justified (for example, chiller capacity being lower than the normal for the species, multiple species and concurrent *Salmonella* sampling obligations exceeding laboratory capability for the sample collection and/or analysis);
d) sampling is also undertaken if the chiller operating conditions (calibration) change significantly;
e) post chill carcasses of cold and warm boning premises are wet/dry swab sampled as per 3.9.1, 3.9.3 and 3.9.4 of post chill carcass;
f) sampling is conducted no later than 24 hours after the onset of chilling; or at the completion of the chilling cycle (if it is less than 24 hours);
g) sampling is conducted in the chilling area itself, prior to wrapping, transportation, freezing, boning or loadout; and
h) post chill carcass description information, as per clause 3.16, is recorded at time of sampling directly into the NMD database or on the sample submission form.

Guidance

- To comply with sampling post chill carcasses within 24 hours from commencement of chilling, the sampling may need to be conducted on carcasses that are warmer than 7°C.

3.7 Sample selection

3.7.1 Sample selection plan

(1) Operators must ensure that:

a) a plan covering the sample selection requirements in accordance with this clause is in place for each species processed where sampling is required; and
b) sampling is conducted according to the plan and the requirements of this notice.

(2) Sample selection must be on either a random or a rotational basis, as specified in clause 3.7, and must include all of the following:

a) processing day, as per clause 3.7.2; and
b) shift, chains, and boning rooms, as per clause 3.7.3; and
c) class, as per clause 3.7.4; and
d) run, as per clause 3.7.5; and
e) time, as per clause 3.7.6.

(3) Operators must keep records relating to sample selection plan.

Guidance

- If multiple species are processed at a premises, a single selected time period to sample all species may be used (same day, shift, and run for all species) if you have enough sample-takers and laboratory personnel to process the samples. Alternatively, a different day of the week or time in the day may be randomly selected for the other species from the remaining days or part days of that week.

3.7.2 Selection of day

(1) Operators must ensure that:

a) all days on which a species is processed are eligible as processing days for selecting samples of that species; and
b) one processing day is randomly selected for sampling, and then the remaining processing days are selected in random order until all processing days of the processing week have been selected; and
c) once all days have been selected, the selection process starts again with all processing days eligible.

Guidance

- For example, if a premises processes Monday - Friday, an initial random selection of 1 of the 5 days is completed, e.g. Wednesday. The next week the random selection will exclude Wednesday and 1 of the 4 remaining days will be selected, and so on. After 5 weeks all processing days will have been selected.

(2) VLT operators who sample carcasses, primal cuts, or bulk meat product must:

a) estimate the number of weeks in which processing will take place that month;
b) randomly select one of those weeks for sampling or, if there is significant uncertainty as to when product will be sent for processing, select the first available processing week of the month for sampling; and
c) randomly select the days in that week, as required by subclause (1), for sampling.

3.7.3 Selection of shifts, chains, and boning rooms

(1) Operators must ensure that rotational sampling occurs for:

a) shifts; and
b) slaughter chains; and
c) boning rooms.

Guidance

- If personnel rotate onto various shifts as a group, this needs to be taken into account in rotational sampling. For example, if there is a red shift and a blue shift, then red shift would need to be sampled both day and night and blue shift would need to be sampled both day and night.

3.7.4 Selection of class

(1) Operators must ensure that rotational sampling for classes occurs, as described below:

a) ovine – 2 classes with no rotation: lamb must be sampled each processing week, except on processing weeks where only sheep are being processed, in which case sheep must be sampled;
b) ratite – 2 classes with rotation: ostrich and emu;
c) cervine – 2 classes with rotation: fallow and ‘other’ (e.g. wapiti, red, hybrid and sika);
d) bovine – 3 classes with rotation: bull, cow and prime; and

e) porcine – 4 classes with rotation: sucklers, porkers, baconers, choppers (due to the relatively infrequent processing of choppers, these must be targeted for sampling when processing occurs to ensure coverage of all classes processed within the month for standard throughput premises or within 4 months for VLT premises).

(2) Operators must not use the proportion of livestock classes in any species processed to deliberately bias the selection of class for that species.

(3) VLT operators must rotate through classes monthly rather than weekly.

Guidance

- The rotational selection of processing day and shift during the processing week and random selection of run and time will permit a cross-section of classes to be sampled during each quarter. The exception to this is porcine choppers, which are selected during 1 processing week each month to ensure coverage.

3.7.5 Selection of run

(1) Operators must randomly select the run for sampling from all runs available that processing day.

3.7.6 Selection of time

(1) Operators must ensure that the time for sampling the initial item is randomly selected.

(2) Random selection must be made by selecting one of the following:

a) a time during the run;
b) a carcass ticket number from the run; or
c) a processing segment of the run no greater than 20 minutes.

(3) The actual sampling time must not vary by more than 10 minutes either side of the originally selected time or, if a processing segment is selected, the actual sampling must be started within that segment.

(4) The actual sampling time must be recorded in a format that can be converted to 24-hour clock units for submission to NMD database.

Guidance

- The time may be chosen by the 24-hour clock system or minutes into the run with a sampling tolerance of 10 minutes either side. For example, 15:33±10 minutes, the sampling period is between 15:23 and 15:43.

  Example of random/rotational selection of day, random selection of run and time for a week with 5 processing days. Random/rotational selection begins again at week 6.

Week Number	**1**	**2**	**3**	**4**	**5**	**6**
Day	**Wednesday**	**Friday**	**Monday**	**Thursday**	**Tuesday**	**Tuesday**
Run	4	3	1	4	2	2
Time (using ticket number)	*4045*	*3125*	*0215*	*3701*	*2245*	*1523*
Time (24-hour clock)	15:33	13:01	08:22	14:45	11:55	10:37
Time (minutes into run)	15	78	61	23	42	36

3.8 Selecting samples

(1) Operators must ensure that samples are selected as follows:

a) the initial item must be randomly selected;
b) the remaining items are selected at time intervals equivalent to the time required to sample; and
c) if the carcass, primal cut or carton of bulk meat available at that time is unsuitable (e.g. carcass goes on to detain rail), then select the next available appropriate carcass, primal cut or carton.

(2) Operators may permit selection of the required number of carcasses, primal cuts or cartons of bulk meat at once from the process and putting them aside to sample at the same time only if there is insufficient product type remaining.

3.8.1 Departure from the original sample selection

(1) If a sample is missed or processing schedules change in a manner that means the original random sampling plan cannot be followed, the operator must ensure that every reasonable effort is made to randomly sample the product type concerned from within the processing time in that processing week to meet the requirements under this notice.

(2) Operators must ensure that any changes in the original sample selection are noted in their own records with the reason for the change.

(3) If insufficient product is available to be sampled at the selected sampling time, an operator must ensure that the required samples are collected from available product in that processing week in the following order of priority:

a) incomplete cartons;
b) from the next run within the shift;
c) multiple product types: bulk 95VL, 65VL, 80VL within a class;
d) different classes;
e) across both shifts within a processing day;
f) from the next day within the processing week.

(4) If the required samples are not collected, this will be considered a “missed” sample.

3.8.2 Collecting additional samples

(1) Operators may take additional samples routinely to allow for practical constraints and potential technical failures.

(2) If additional samples are taken, operators must ensure that:

a) the original NMD selection and the additional sample(s) are identified; and
b) the results are entered in the NMD database in the following order:

i) the original NMD samples; and
ii) the additional sample(s), if necessary, to give the required total of results over 1 processing week.

3.9 Sampling equipment

(1) Operators must ensure that the sample-taker has appropriate sampling equipment.

Guidance

General sampling equipment includes:

a) a means to record sampling details;
b) a method to label vials;
c) spare sterile templates, vials, swabs, and gloves if necessary;
d) insulated containers with ice packs (slicker pads or bags of shaved ice);
e) ladders or step-stools for access to sampling sites if necessary;
f) ethanol/iso-propanol wipes if required for sterilising templates;
g) knife (or scalpel) and bags for bulk sampling; and
h) swabbed site sampling equipment:
    i) templates as specified in clause 3.10;
    ii) if using sterile vials without diluent, peptone for wetting swabs;
    iii) volume of diluent required (either included at time of sampling or later at time of testing);
    iv) sterile cotton-tipped swabs; and
    v) for composite *Salmonella* sampling of carcasses, ssBPW for wetting swabs.

3.10 Templates and swabs for swab sampling

3.10.1 Templates

(1) Operators must ensure that templates to delineate the site to be swabbed are used for sampling in accordance with this clause.

(2) Templates must be made of one of the following inert materials capable of delineating the correct sized sample area:

a) stainless steel rod (made into circular or square hoops with handle);
b) flexible material (plastic or cardboard); or
c) flat, rigid sheet metal for 5 cm^2 or 25 cm^2 templates.

(3) The template must conform to the area measurements for the area to be sampled for each species listed in Table 4:

Table 4

Species		**Ovine, caprine**	**Bobby calf, cervine, ratite, and porcine**	**Bovine**
Area to be sampled		5cm^2	25cm^2	100cm^2
Area measurements*	**Circular – diameter**	25.2mm	56.4mm	112.8mm
	Square – side	22.4mm x 22.4mm	50.0mm x 50.0mm	100.0mm x 100.0mm

*NOTE: The tolerance is ±1.1mm for circular templates and ±1.0mm for square templates

Guidance

- See Chapter 5, Section 5.4 Swab Sample Template Calibration in MIMM for further information.

(4) Operators must ensure that the dimensions of all templates used for sampling are calibrated at least once every 6 months and results are recorded in their equipment records.

(5) Templates must be sterile when used for sampling.

(6) Sterilisation can be achieved by using:

a) pre-sterilised (autoclaved) multiple templates;
b) ethanol/iso-propanol based disinfectant wipes; or
c) 70% ethanol for flame sterilisation.

3.10.2 Swabs

(1) Operators must ensure that swabs used for sampling are sterile.

(2) Sterilisation can be achieved by:

a) autoclaving; or
b) purchasing pre-sterilised swabs.

(3) If commercial swabs supplied in individual plastic tubes are used and the swabs are returned to those tubes after sampling, the laboratory must ensure that the suspension buffer required for each site sampled is used to rinse out the inside of all the tubes of swabs associated with that site to remove any bacteria that may have been smeared onto the inner walls by the swabs during sampling and transport.

3.11 Diluents

(1) An operator's laboratory must ensure that diluents are prepared, labelled, and provided to the operator in accordance with this clause.

(2) Sterile diluents used for sampling and testing must not be cloudy, loose lidded, odorous, or have passed their expiry dates.

(3) The operator must ensure that the sample-taker uses the appropriate diluent according to whether antibacterial agents, such as chlorinated compounds, were applied to the product prior to sampling or not.

3.11.1 Peptone diluent

(1) Peptone diluent, which is used for moistening of swabs for collection of carcass samples for APC and generic *E. coli* and primal cut samples for APC and generic *E. coli*, must be prepared with the composition set out in Table 5, and be sterilised:

Table 5

0.1% Peptone Diluent	Amount
Peptone	1.0g
Sodium chloride (NaCl)	8.5g
Distilled water	1000ml

3.11.2 Single strength buffered peptone water (ssBPW)

(1) ssBPW, which is used for moistening swabs for collection of carcass samples for *Salmonella* and for primal cut samples for *Salmonella,* APC, and generic *E. coli*, must be prepared with the composition set out in Table 6, and be sterilised.

Table 6

ssBPW Diluent	Amount
Peptone	10.0g
Sodium chloride (NaCl)	5.0g
Disodium phosphate (Na_2HPO_4)	3.5g
Potassium phosphate (KH_2PO_4)	1.5g
Distilled water	1000ml
Final pH 7.2±0.2 at 25ºC	

3.11.3 Double strength buffered peptone water (dsBPW)

(1) dsBPW, which is used for composite *Salmonella* primal cut swabs and bulk meat samples, must be prepared with the composition set out in Table 7, and be sterilised.

Table 7

dsBPW Diluent	Amount
Peptone	20.0g
Sodium chloride (NaCl)	10.0g
Disodium phosphate (Na_2HPO_4)	7.0g
Potassium phosphate (KH_2PO_4)	3.0g
Distilled water	1000ml
Final pH 7.2±0.2 at 25ºC	

3.11.4 Antibacterial agents

(1) Operators must inform the laboratory if antibacterial agents, such as chlorinated compounds, are used during processing so that the laboratory can prepare the appropriate diluent.

(2) If antibacterial agents are used, the laboratory must:

a) determine suitable non-antimicrobial neutralising additives;
b) add them to the diluent; and
c) label the mixture accordingly.

Guidance

- For example, if a chlorinated compound, such as acidified sodium chlorite (ASC) has been applied prior to sampling, the laboratory must ensure that a peptone diluent with the addition of 1.0ml of a 3% sodium thiosulphate ($Na_2S_2O_3$) solution to 1 litre of diluent is used for sampling.

3.12 Sample collection

3.12.1 APC and *E. coli*

(1) Operators must ensure that the template sizes, swabs, and diluents in Table 8 are used for APC and generic *E. coli* sampling of the species listed:

Table 8

Species	Ovine, caprine	Bobby calf, cervine, porcine, and ratite	Bovine
Template size	5cm^2	25cm^2	100cm^2
Number of swabs per carcass site or primal cut site	2 swabs: 1 wet,1 dry	4 swabs: 2 wet, 2 dry	6 swabs: 3 wet, 3 dry
Volume of peptone diluent	10ml	15ml	15ml

3.12.2 Salmonella

(1) For *Salmonella* sampling for carcasses, the operator must ensure a separate set of sites is swabbed from the opposite side of the carcass from APC/generic *E. coli* samples.

Guidance

- A separate set of primal cut swabs is not required for *Salmonella*. The laboratory will use remaining sample diluent after APC/generic *E. coli* analysis.

(2) The following template sizes, swabs (which may be placed into single volume vials or composited into a single bottle), and diluents must be used for *Salmonella* sampling the species listed in Table 9.

Table 9

Species	Bovine	Bobby calf	Caprine	Ratite
5 carcass composite	**3 sites per carcass**	**3 sites per carcass**	**3 sites per carcass**	**1 site per carcass**
Template (1 per site)	100cm^2	25cm^2	5cm^2	25cm^2
Total number of swabs	6 swabs x 3 sites x 5 carcasses = 90 swabs	4 swabs x 3 sites x 5 carcasses = 60 swabs	2 swabs x 3 sites x 5 carcasses= 30 swabs	4 swabs x 1 site x 5 carcasses = 20 swabs
ssBPW diluent sample volume	250ml	150ml	75ml	75 ml
Bottle size	500ml	250ml	250ml	250ml

3.13 Carcass sample sites

(1) The operator must ensure that carcasses are sampled in accordance with all the provisions of this clause.

(2) If more than one site per carcass (as specified in this clause) must be sampled, on each carcass samples must be collected from one side of the carcass for APC/generic *E. coli* and from the other side of the carcass for *Salmonella* if *Salmonella* testing is required.

(3) Sampling should alternate between leading and trailing sides.

Guidance

- See Chapter 5 Collection and Preparation of Samples in MIMM for photographs of carcass sample sites.

3.13.1 Bovine carcass sample sites

(1) The following 3 sites must be swabbed on bovine:

a) Rump: The site is centred on the fascia overlying the semitendinosis muscle (the 'eye round'). The centre of the sampling site is halfway along the muscle on its superficial lateral margin. Lateral to semimembranosis is the gluteobiceps muscle (the 'outside flat') and the 100cm^2 template may overlap both muscles;
b) Flank: The site is centred on the fascia overlying the cutaneous trunci muscle. The centre of the site is 10cm lateral to the umbilicus on a line drawn along the ninth rib (count from the head end) from the spine and continued on to the cut edge of the abdominal wall; and
c) Brisket: The site is centred 10cm off the central midline. The lower edge of the sampling site is an imaginary line drawn transversely along the thoracic wall at the level of the point of the elbow. The margins of the sampling site may overlap onto the fascia overlying the caudal margin of the pectoral profundis muscle.

3.13.2 Ovine carcass sample sites

(1) The following carcass site must be swabbed on ovine:

a) Forequarter opening Y-cut: The Y-cut site is centred on the anterior aspect of the humero-radial (elbow) joint close to the brachial vein, which is clearly visible at the site and may be a little erratic in its course.

3.13.3 Caprine carcass sample sites

(1) The following 3 carcass sites must be swabbed on caprine:

a) Outside hindleg: The outside hindleg site is centred one third up on a vertical line originating at the midpoint of a line between the ischial crest and stifle, and extending to a line horizontal to the cut end of the hock; and
b) Flap: The flap site is centred ~50mm from the flap edge, midway between the flap joint and the xiphoid cartilage; and
c) Forequarter opening Y-cut: The Y-cut site is centred on the anterior aspect of the humero-radial (elbow) joint close to the brachial vein, which is clearly visible at the site and may be a little erratic in its course.

3.13.4 Bobby calf carcass sample sites

(1) The following 3 carcass sites must be swabbed on bobby calves:

a) Fore rump: Centred on a horizontal line dissecting the forward (lower) edge of the rectal canal, in line with a cut tail, and no greater than 50mm horizontally from the edge of the rectal canal;
b) Flank: The site is centred ~50mmm from the flank edge, midway between the flank joint and the xyphoid cartilage; and
c) Foreleg: Centred on the outside surface of the foreleg, on the lateral head of the triceps brachii.

(2) The side (left or right of the animal) of the carcass that APC/generic *E. coli* swabs are collected from must be recorded.

3.13.5 Cervine carcass sample sites

(1) On traditionally dressed cervine, the following 3 carcass sites must be swabbed:

a) Posterior hindleg: The hindleg sample site is centred longitudinally and laterally on an imaginary line drawn between the posterior aspect of the aitch bone and the Achilles tendon;
b) Sternum: The sternum site is centred immediately adjacent to the intersect of the abdominal cavity opening and brisket, but outside the area of "standard brisket trim" (by <10mm at its closest edge). Note: Tissue exposed by the standard trim must not be sampled; and

c) Foreleg: The foreleg site is centred on the anterior (forward) aspect of the humero-radial (elbow) joint close to the brachial vein, which is clearly visible at the site (a little erratic in its course).

(2) On inverted dressed cervine, the following 3 sites must be swabbed:

a) Inside hindleg: The sample site is centred on the medial proximal of the hindleg immediately adjacent to the pelvic symphysis (midline);
b) Brisket: The brisket sample site is centred longitudinally on the brisket, immediately outside the area of "standard brisket trim" (by <10mm at its closest edge). Note: Tissue exposed by the standard trim must not be sampled; and
c) Foreleg: The foreleg site is centred on the anterior (forward) aspect of the humero-radial (elbow) joint close to the brachial vein, which is clearly visible at the site (a little erratic in its course).

3.13.6 Porcine carcass sample sites

(1) The following 3 carcass sites must be swabbed on porcine:

a) Outside hindleg: Is centred midway between the stifle and hip joint;
b) Lower flap: Is centred midway between the 2 lower nipples, 50mm from the abdominal incision; and
c) Outside shoulder: Is centred above the anterior (forward) top end of the shoulder blade.

3.13.7 Ratite (leg hung) carcass sample sites

(1) The following carcass site must be swabbed on ratite:

a) Inside leg: Is centred longitudinally on the opening cut line and laterally where the edge of the remaining flap contacts the leg. It is recommended that this site be alternated between left and right legs.

3.14 Wet and dry swabbing technique

(1) Operators must ensure that wet swabbing and dry swabbing is done in accordance with this clause.

(2) Wet swab sampling must be done by:

a) placing the template in the correct position and ensuring the template does not move;
b) moistening swabs for 5 seconds in the diluent;
c) rubbing the wet cotton portion of the swabs vertically, horizontally, and diagonally across the entire surface bounded by the template, using as much force as possible without breaking the shaft of the swab;
d) rotating the swabs to ensure the entire bud is in contact with the surface for:

i) 10 seconds swabbing time for ovine, caprine 5cm^2 templates; and
ii) 20 seconds swabbing time for bobby calf, cervine, porcine, and ratite 25cm^2 templates and bovine 100cm^2 templates.

(3) Dry swab sampling must be done immediately after wet swabbing in the same manner as described in (2) c) - d) by rubbing the same area with dry swab/s until the area being sampled is dry.

(4) Once the wet or dry swabbing is complete:

a) insert all swabs together into a transport vial, which may or may not contain the diluent; and
b) place the sample in an insulated container, avoiding direct contact with the ice pack.

(5) If contamination occurs during sampling, the sample must be discarded and a new sample taken from a new item.

Guidance

- If 1 of the 3 carcass site samples is contaminated, all 3 samples must be replaced so that they are all from the same carcass.

3.15 Whole tissue composite sampling technique for bulk meat

(1) Operators must ensure that whole tissue composite sampling for bulk meat is done in accordance with this clause.

(2) Whole tissue composite sampling must be conducted aseptically and according to the following procedures:

a) collect 5 whole tissue samples by trimming a thin layer from the surface;
b) ensure each sample is approximately 10 grams in weight to result in at least 50 grams collected per carton;
c) collect samples from the 4 diametrically opposite corners of the carton and one from the centre of the carton;
d) deposit each of the 5 samples per carton into a sterile sample bag; and
e) seal the sample bag.

(3) Collect samples from an original external carcass surface if possible. If not available, then a cut surface may be sampled.

Guidance

- See Chapter 5 Collection and Preparation of Samples in MIMM for diagrams of carton sample sites.

(4) If contamination occurs during sampling, the sample must be discarded and a new sample taken.

Guidance

- A new sample should be taken either from the same piece of meat, provided there is sufficient external surface available, or from a different piece of meat from within the same quadrant from the same carton. If this cannot be achieved, all samples from that carton must be discarded and a new carton must be selected, and a full set of samples collected from the new carton.

3.16 Sample description information

(1) Operators must ensure that the following sample description information is recorded directly into the NMD database prior to transport of the sample to the laboratory or, by prior arrangement with the laboratory, recorded on a sample submission form and sent to the laboratory with the samples:

a) sample-taker's name;
b) premises from which samples were taken;
c) sampling date;
d) time (24 hour clock) that sampling began, which is the time of sampling of the first item in the sample set;
e) run, shift, species, and class; and
f) for carcasses: side sampled (leading or trailing);
g) for primal cuts and bulk meat product: descriptors of the boning process (cold, warm, hot);
h) for post chill product: descriptors of the boning process (cold or warm), chiller ID, and number of hours product had been chilled prior to sampling.

3.17 Storing and transporting samples

(1) Operators must ensure that samples are stored and transported in accordance with this clause.

(2) All samples must be kept above 0°C and below 10°C for the entire time between sampling and receipt by the laboratory.

(3) Hot/warm boned whole tissue samples must be rapidly cooled to <10°C but not frozen and kept as per clause (2).

Guidance

- Ideally samples should be reduced to <5°C to reduce the possibility of microbiological growth.
- Premises may choose to have samples transported to an on-site laboratory for some analyses and off-site for others according to schedules, laboratory scope of testing and other factors.

3.18 Receipt of samples by laboratories

(1) Laboratories must ensure that the NMD testing of samples is initiated within 30 hours after collection of the first sample of the sample set.

(2) A laboratory must treat a sample as unsuitable for NMD testing, reject that sample, and seek replacement samples in that processing week from the operator if:

a) samples arrive too late for testing to commence within 30 hours of time the first sample of the set was taken;
b) samples are received less than 0°C;
c) samples received exceed 10°C; or
d) samples were not taken by a sample-taker whose details are held by the laboratory.

(3) Laboratories must enter the sample description information supplied by the sample-taker on the sample submission form into the NMD database if not already entered by the sample-taker.

(4) Laboratories must record the following sample receipt details:

a) confirmation that the person who took the sample is a sample-taker whose details are held by the laboratory;
b) the sample temperature;
c) the time from sampling to initiation of analysis; and
d) confirmation that the sample is suitable for testing.

3.19 Sample dilutions

(1) The swab suspension (swab immersed in the initial volume of 10ml or 15ml diluent) is an undiluted sample (the zero dilution) and must be entered into the calculations as the 10^0 dilution.

(2) Laboratories must ensure that:

a) the volume of diluent used:

i) is sufficient to enable inoculation of duplicate agar plate count plates, half plates, or Petrifilm™; and
ii) if samples of primal cuts are tested, there is sufficient volume remaining for the composite *Salmonella* pre-enrichment broth; and

b) the bacteria are evenly distributed within the diluent, such as by shaking the sample.

(3) To avoid having a "too numerous to count" (TNTC) result, laboratories must ensure that sufficient dilutions are undertaken to obtain plate counts below the maximum allowable counts on at least one, or one set of, plates as follows:

a) for APC[30], 10^0 to 10^{-4} plus higher dilutions as required; and
b) for generic *E. coli*, 10^0 to 10^{-3} plus higher dilutions as required.

3.19.1 Duplicate plating

(1) To give statistical confidence to the results, duplicate plating must be carried out for all dilutions prepared for:

a) Aerobic Plate Count (APC) including Spiral Plater method; and
b) APC and *Escherichia coli* Petrifilm™.

(2) If a laboratory can demonstrate analytical precision, then singlet plating can be undertaken for all dilutions (the undiluted sample must be plated in duplicate).

Guidance

- See Chapter 5, Appendix 1 Important Points for Accurate Microbiological Analysis and Chapter 2 Quality Control in Microbiology Laboratories in MIMM for further information on Analytical Precision.

3.19.2 Allowable counts

(1) Laboratories must ensure that only plates with colonies that fall in the acceptable counting range as specified in Table 10 are counted:

Table 10

Plate dilution	Spreadplate APC	Spreadplate APC ½ plate	Petrifilm™ APC	Petrifilm™ *E. coli*
10^0	0-300	0-150	0-250	0-150
All other dilutions	30-300	15-150	25-250	15-150

3.19.3 High end dilutions and TNTC results

(1) If a count exceeds the maximum allowable count given in Table 10, on the highest dilution, TNTC must be recorded.

(2) The test does not need to be repeated for the sample with a TNTC result.

(3) If a TNTC result occurs, a laboratory must extend the dilution series to ensure results are within the acceptable counting range for subsequent samples.

3.20 Sample preparation

3.20.1 Swabs for APC and generic *E. coli* testing

(1) Laboratories preparing swabs for APC and generic *E. coli* testing must ensure that:

a) if the swab samples arrive in vials without beads, 4-9 beads are added to each vial;
b) if the swab samples arrive in vials with no diluent, the correct volume of diluent is added as follows:

i) for bovine, bobby calf, cervine, porcine, and ratite: 15ml of 0.1% peptone diluent; and
ii) for ovine and caprine: 10ml of 0.1% peptone diluent; and

c) the vial is closed and shaken vigorously to loosen the cotton bud of the swab for 2 minutes by either:

i) shaking by hand; or
ii) using a combination of initial shaking by hand and a vortex mixer in 3 x 10 second bursts; and

d) the cotton swabs are not removed from the vials at any stage prior to the completion of plating.

3.20.2 Bulk meat samples for APC and generic *E. coli* testing

(1) Laboratories preparing bulk meat (whole tissue) samples for APC and generic *E. coli* testing must ensure that:

a) the whole tissue samples (5 x ~10g) are placed on a sterile chopping board and finely sliced aseptically;
b) 25g of sliced tissue is aseptically weighed directly into a sample bag;
c) 225ml of sterile diluent (0.1% peptone + 0.85% NaCl) is added to the bag; and
d) the bag is stomached for 2 minutes.

3.21 APC test methods

(1) Laboratories must ensure that all APC tests are conducted in accordance with this clause.

(2) APC testing must use one of the following methods:

a) APC[30] by spread plate, as described in clause 3.21.5; or
b) APC Petrifilm™ Aerobic Plate Count, as described in clause 3.21.6; or
c) APC[30] spiral plater, as described in clause 3.21.7.

(3) All quality control procedures for the media and methods must be carried out using *Pseudomonas aeruginosa* NZRM 981 or *Weissella viridescens* NZRM 3313 as the positive control.

3.21.1 Preparing plate count agar plates

(1) All plate count agar plates to be used for spread or spiral plating must be air dried if necessary to ensure rapid absorption of the sample volume use by one of the following methods:

a) incubation for 24h at 30-37°C, inverted with lids in place;
b) inverting with lids ajar in a laminar flow cabinet for 20-30 minutes;
c) inverting with lids ajar in a still air incubator at 30-37°C for 1½ to 2 hours; or
d) inverting with lids ajar in a forced air incubator at 30-37°C for 30 minutes.

3.21.2 Initiation of analysis

(1) Initiation of analysis for APC starts when the sample is suspended and ready for serial dilution.

3.21.3 Incubation

(1) The required incubation temperature for the APC is 30°C ± 1°C (ISO 17604:2015).

(2) After the required 48-hour (h) incubation time for APC, count colonies and record results.

(3) If colonies are indeterminate at 48h, estimate a count, re-incubate for a further 18-24h, recount and record results.

(4) If for some reason plates cannot be counted at 48h, plates may be:

a) incubated for up to 72h in total; and
b) removed from the incubator and stored for no longer than 48h in a refrigerator at 0°C to 5°C.

(5) The total incubation time must be recorded on the result sheet.

(6) Any additional storage time of the plates following incubation must be recorded on the result sheet.

3.21.4 APC results

(1) Count only plates within the acceptable counting range defined in Table 10.

(2) Record the result as the number of colony forming units (CFU)/cm^2 or /g of sample.

(3) If there are no colonies present on both of the duplicate 10^0 dilution plates, record the result as "not detected".

3.21.5 APC^{30} by spread plate method

(1) The following is the APC^{30} by spread plate method:

a) dispense 0.1ml (100µl) volumes of each dilution onto 2 count agar plates, or a single ½ plate;
b) mix the dilutions thoroughly and start plating with the highest dilution;
c) spread the inoculum over the surface of the agar as evenly and as quickly as possible using a sterile spreader;
d) allow the inoculum to soak into the agar surface (should occur within 15 minutes); and
e) invert the plates so that the agar is on the top and incubate.

Guidance

- The same pipette/tip may be used for all inoculations when plating from the highest to lowest dilution for APC spread plates.
- If liquid does not soak in (i.e. the plates were not dried sufficiently), the viable bacteria present in the inoculum may start to replicate and spread, possibly resulting in an inaccurate count.
- Inversion prevents condensation dropping onto the surface of the agar, reducing contamination and preventing the spread of motile micro-organisms.

3.21.6 APC^{30} Petrifilm™ Aerobic Plate Count method

(1) The following is the APC^{30} Petrifilm™ Aerobic Plate Count method:

a) process each Petrifilm™ plate individually;
b) place the Petrifilm™ Aerobic Plate Count plate onto a flat surface, label and lift the top film; and
c) dispense 1ml volumes of each dilution onto the agar in duplicate;
d) slowly roll the top clear plastic film down onto the inoculum to prevent entrapment of air bubbles;
e) distribute the sample evenly using the spreader provided (ridge side down as the plates do not have a foam dam), by applying gentle even pressure;
f) move the completed Petrifilm™ plate to one side and leave for 1 minute for the agar to set; and
g) incubate all Petrifilm™ plates clear side up in stacks of no more than 20.

3.21.7 APC^{30} Spiral plater method

(1) For the APC^{30} spiral plater method, plates must be prepared according to the manufacturer's guidelines supplied with the spiral plater and the plate surface must be smooth.

(2) The following is the APC^{30} spiral plater method:

a) follow all manufacturer's instructions for use;
b) after inoculation, leave the plates on the bench right-way-up for 10 minutes to allow the inoculum to soak into the agar; and
c) invert the plates and incubate.

3.22 *Escherichia coli* Petrifilm™ test method

(1) Laboratories must use the *E. coli* Petrifilm™ method in accordance with this clause.

(2) All quality control *E. coli* testing procedures for the media and methods must be carried out using:

a) *Escherichia coli* NZRM 916 as the positive control; and
b) *Klebsiella pneumoniae* NZRM 482 or *Klebsiella aerogenes* NZRM 798 as the negative control.

3.22.1 Initiation of analysis

(1) Initiation of analysis for *E. coli* commences when the sample is suspended and is ready for serial dilution.

3.22.2 Method

(1) Place a Petrifilm™ *E. coli* plate on a flat surface and hold the plate flat.

(2) Mix the dilutions thoroughly before dispensing.

(3) Lift the top film and dispense 1ml volumes of each dilution onto the agar in duplicate. Do not inoculate several agars at the same time. It is imperative to inoculate and lower the top film before proceeding to the next.

(4) Slowly roll the top clear plastic film down on to the inoculum to prevent entrapment of air bubbles.

(5) If necessary, use the spreader provided flat side down, as Petrifilm™ *E. coli* plates have a rim around the well to distribute the sample evenly by applying gentle even pressure to the spreader.

(6) Move the completed Petrifilm to one side and leave for 1 minute for the agar to set.

(7) Incubate all plates for 18-24h at 35 ± 1°C or 37 ± 1°C clear side up and in stacks of no more than 20. Count colonies and record results.

(8) If colonies are indeterminate at 24h, count as best as possible, re-incubate for a further 18-24h and recount. Record the total incubation time on the result sheet.

(9) If for some reason plates cannot be counted after the 18-24h incubation period, the plates may be removed from the incubator and stored for no longer than 72h in a refrigerator at 0° - 5°C. Record the storage time and temperature on the result sheet.

(10) Count as generic *E. coli* all blue colonies with or without gas bubbles.

(11) Count only plates with between 15 to 150 colonies for Petrifilm™ *E. coli* plates. (For 10^0 dilution, <15 may be counted.) Do not count colonies that have grown on the foam dam.

(12) High numbers of generic *E. coli* will turn the medium blue and high numbers of Enterobacteriaceae will turn the medium red. Both situations make accurate enumeration impossible. Should this occur for the highest dilution plated, record results as TNTC and immediately notify the operator.

3.22.3 Generic *E. coli* results

(1) Express the results as the number of generic *E. coli* CFU/cm^2 or /g of sample.

(2) If blue colonies with or without gas are not detected, record the results as “not detected”.

3.23 *Salmonella* test method

(1) Laboratories must use the method in accordance with clause 3.23 for *Salmonella* detection.

(2) All quality control procedures for the media and methods must be carried out using:

a) *Salmonella Menston* NZRM 383 as the positive control; and
b) *E. coli* NZRM 916 as the negative control.

3.23.1 Initiation of analysis

(1) Initiation of analysis for *Salmonella* commences when the sample suspension is placed in the incubator.

3.23.2 Preparing composite samples for *Salmonella* testing

(1) All swabs used for *Salmonella* sampling of NMD carcass sites must be placed into a single sterile bottle allowing sufficient volume for diluent and mixing.

(2) The listed amount of diluent must be added for each species in Table 11:

Table 11

Species	Bovine	Bobby calf	Caprine	Ratite
Sites per carcass	3 sites per carcass	3 sites per carcass	3 sites per carcass	1 site per carcass
Total number of swabs	6 swabs x 3 sites x 5 carcasses = 90 swabs	4 swabs x 3 sites x 5 carcasses = 60 swabs	2 swabs x 3 sites x 5 carcasses= 30 swabs	4 swabs x 1 site x 5 carcasses = 20 swabs
Volume of ssBPW diluent	250ml	150ml	75ml	75ml
Bottle size	500ml	250ml	250ml	250ml

(3) Add an appropriate number of glass beads to aid suspension of the microbes in the diluent, close the bottle and mix vigorously for 2 minutes.

(4) Prepare composite samples for *Salmonella* testing:

a) for primal cut, composite the swab suspensions remaining after generic *E. coli* and APC plating has been completed; and
b) for bulk meat, composite *Salmonella* samples by adding an equal volume of dsBPW to 5ml of composite sample suspension (5 x 1ml of each sample suspension) to make up a final *Salmonella* sample suspension.

3.23.3 Pre-enrichment incubation

(1) Immediately after preparing the final *Salmonella* sample suspension by addition of dsBPW or suspension of carcass swabs in ssBPW, incubate the pre-enrichment sample at 35±1°C or 37±1°C for 18-24 hours.

(2) Once the *Salmonella* analysis has been initiated, all steps must be completed within the time and temperature requirements, and the pre-enrichment sample and/or Rappaport-Vassiliadis *Salmonella* (RVS) selective enrichment broth must not be refrigerated at any time.

3.23.4 Enrichment

(1) Following incubation, transfer 0.1ml of the pre-enrichment sample to 10ml of RVS selective enrichment broth pre-warmed to 42°C.

(2) Incubate the RVS selective enrichment broth for 24 hours ±2 hours at 42±0.2°C in a water bath or incubator.

3.23.5 Plating

(1) Following incubation, transfer a loopful (10µl) of the RVS enrichment culture to one plate of Brilliant Green Modified (BGM) agar and to a plate of Xylose Lysine Desoxycholate (XLD) agar selective plating media and streak to obtain single, well isolated colonies. Label plates with the agar type (BGM

or XLD) as the agar colour can change during incubation making it hard to distinguish between the 2 types of media.

(2) Incubate both agar plates for 18-24 hours at 35±1°C or 37±1°C.

(3) Examine the agar plates for the presence of typical *Salmonella* colonies:

a) BGM agar: pink colonies surrounded by bright red medium; and
b) XLD agar: red colonies with a black centre (H_2S negative serotypes have red colonies without a black centre).

3.23.6 Confirming presumptive positive *Salmonella*

(1) Select 5 or more single typical *Salmonella* colonies from the XLD and/or BGM selective plating media for confirmation.

(2) Use biochemical tests and/or commercial kits as listed in MIMM Identification of *Salmonella* Colonies, including serotyping, test each of the 5 colonies sequentially.

(3) If any one of these colonies is positive, the sample is deemed positive.

(4) Upon detection of a positive colony, any remaining colonies need not be tested.

3.23.7 Final confirmation and serological typing

(1) Streak a positive colony from either selective media onto MacConkey agar (without salt and crystal violet).

(2) Incubate for 24h at 35±1°C or 37±1°C.

(3) Subculture any typical colourless colonies to plate count agar and incubate overnight at 35±1°C or 37±1°C.

(4) Following overnight incubation, colonies must be submitted to the Institute of Environmental Science and Research (ESR) Enteric and *Leptospira* Reference Laboratory, Wallaceville directly.

3.23.8 *Salmonella* results

(1) If samples are composited for *Salmonella* testing, express the result as *Salmonella* "detected in a composite sample" or "not detected in a composite sample".

3.24 Reporting and recording results

(1) Laboratories must report test results for the purpose of this Notice using:

a) automated result calculation (NMD database entry); or
b) manual result calculation followed by entry into the NMD database.

Guidance

- The NMD database takes raw counts and dilution data and automatically performs all calculations, providing descriptive statistics, and graphical profiles. It acts as an ongoing repository of data derived from the NMD sampling programmes. It will also accept partially calculated CFU/ml, CFU/g or fully calculated log CFU/ml, log CFU/g results.

3.24.1 Automated result calculation

(1) Laboratories that use the NMD database to enter results of APC or *E. coli*, or the presence/absence results in the case of *Salmonella,* must do so as per subclauses (2) and (3).

(2) Data must be entered into the NMD database as follows:

a) test results must be entered by the laboratory; and
b) confirmation results for *Salmonella* must be entered by ESR.

(3) Prior to submitting results to the NMD database, results must be authorised as follows:

a) samples: the type of sample, the run, shift, and time of day (24-hour clock) must be authorised by a person who is able to confirm that a sample-taker whose details are held by the laboratory took the samples;
b) laboratory test results must be entered by a person who must authorise the report upon confirming that:

i) a sample-taker whose details are held by the laboratory took the samples; and
ii) that a KTP for the tests concerned has signed the laboratory report; and

c) the confirmed result and serotyping for *Salmonella* must be entered by ESR on confirmation that the report has been signed by a competent ESR analyst.

3.24.2 Manual result calculation

(1) Laboratories that use manual result calculation of APC or generic *E. coli*, or the presence/absence results in the case of *Salmonella*, must do so in accordance with clause 3.24.2.

(2) The dilution series values for calculations below are to be mathematically expressed as 10^0, 10^{-1}, 10^{-2} and so on.

> **Guidance**
> - The swab suspension is not a dilution and is entered into calculations as the 10^0 dilution.
> - Although the whole tissue suspension is a 1:10 dilution, in order to maintain consistency in plate labelling it is entered into calculations as the 10^0 dilution or zero dilution.

(3) Calculate the number of CFU per cm^2 or per g using the following rationale.

Aerobic plate count (spread plate)

Swab samples – 100cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{0.1\text{ml}} \times \frac{15\text{ml}}{100\text{cm}^2}$$

Swab samples – 25cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{0.1\text{ml}} \times \frac{15\text{ml}}{25\text{cm}^2}$$

Swab samples – 5cm^2 area and 10ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{0.1\text{ml}} \times \frac{10\text{ml}}{5\text{cm}^2}$$

Whole tissue samples – 25g sample + 225ml diluent = 250ml total volume

$$\text{CFU / g} = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{0.1\text{ml}} \times \frac{250\text{ml}}{25\text{g}}$$

Aerobic plate count (Petrifilm™)

Swab samples – 100cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{15\text{ml}}{100\text{cm}^2}$$

Swab samples – 25cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{15\text{ml}}{25\text{cm}^2}$$

Swab samples – 5cm^2 area and 10ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{10\text{ml}}{5\text{cm}^2}$$

Whole tissue samples – 25g sample + 225ml diluent = 250ml total volume

$$\text{CFU / g} = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{250\text{ml}}{25\text{g}}$$

Aerobic plate count (spiral plater)

Calculate the number colony forming units per cm^2 or per g using the procedures outlined in the spiral plater's operating instructions. The typical volume for the inoculum is 50µl = 0.05ml or 100µl = 0.1ml.

Swab samples – 100cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Inoculum ml}} \times \frac{15\text{ml}}{100\text{cm}^2}$$

Swab samples – 25cm^2 area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Inoculum ml}} \times \frac{15\text{ml}}{25\text{cm}^2}$$

Swab samples – 5cm^2 area and 10ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Inoculum ml}} \times \frac{10\text{ml}}{5\text{cm}^2}$$

Whole tissue samples – 25g sample + 225ml diluent = 250ml total volume

$$\text{CFU / g} = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{\text{Inoculum ml}} \times \frac{250\text{ml}}{25\text{g}}$$

Escherichia coli (Petrifilm™)

Swab samples – 100cm² area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{15\text{ml}}{100\text{cm}^2}$$

Swab samples – 25cm² area and 15ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{15\text{ml}}{25\text{cm}^2}$$

Swab samples – 5cm² area and 10ml suspension volume

$$\text{CFU / cm}^2 = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{10\text{ml}}{5\text{cm}^2}$$

Whole tissue samples – 25g sample + 225ml diluent = 250ml total volume

$$\text{CFU / g} = \frac{\text{Count 1 + Count 2}}{2} \times \frac{1}{\text{Dilution}} \times \frac{1}{1\text{ml}} \times \frac{250\text{ml}}{25\text{g}}$$

3.25 Reporting exceptions

3.25.1 Periods of not operating

(1) An operator must notify the laboratory in writing if they are not operating within a processing week (standard throughput premises) or month (VLT premises).

(2) The laboratory must record "not operating" in the NMD database when notified by the operator.

3.25.2 Missed samples

(1) An operator must notify the laboratory in writing if any samples are missed.

(2) The laboratory must enter a "missed sample" result in the NMD database when notified by the operator.

3.25.3 Technical failures

(1) If a sample was taken, but a technical failure occurs at the laboratory, the laboratory must enter the details of the sample and the nature of the technical failure.

3.26 Limits of detection for APC and *E. coli*

(1) Laboratories must ensure that results are entered in accordance with clause 3.26.

(2) If there is not a count within the acceptable range as per Table 10, then:

a) if colonies are not present, enter results as "not detected"; or
b) if colonies are too numerous to count, enter results as "TNTC".

(3) Not detected results are results less than the lowest limit of detection.

(4) The values used to represent the lowest limit of detection and "not detected" results in the NMD database are as set out in Table 12.

Table 12

Volume, Area of Site, Weight of Sample	**Lowest limit of detection (LLD) Value**				**"Not Detected"**
	APC CFU/cm² or /g	**APC Log_{10} value**	***E. coli* CFU/cm² or /g**	***E. coli* Log_{10} value**	**Log_{10} value**
Spreadplate 0.1ml			Not applicable	Not applicable	
5cm²	10	1.00			-0.31
25cm²	3	0.48			-0.53
100cm²	0.75	-0.12			-1.13
Bulk Meat (25g)	50	1.70			-0.31
Petrifilm™ 1ml					
5cm²	1	0.00	1	0.00	-0.31
25cm²	0.3	-0.52	0.3	-0.52	-0.53
100cm²	0.075	-1.12	0.075	-1.12	-1.13
Bulk Meat (25g)	5	0.70	5	0.70	-0.31
Spiral plater 50µl			Not applicable	Not applicable	
5cm²	20	1.30			-0.31
25cm²	6	0.75			-0.53
100cm²	1.5	0.18			-1.13
Bulk Meat (25g)	100	2.00			-0.31

Guidance

- When CFU/cm² results (arithmetic) are transformed to log CFU/cm² results (logarithmic) for NMD reporting all results above the lowest limit of detection, even though they may be negative logarithmic values, they represent detections/counts if greater than the values in the above table.

3.27 National Microbiological Criteria

3.27.1 M-limits

(1) The regulatory limits set out in Table 13 are referred to as the M-limits for APC and for generic *E. coli* for the relevant species.

Table 13

Species	**M-limit (APC log_{10})**	**M-limit (generic *E. coli* log_{10})**
Bovine	4.00	2.00
Bobby calf	5.00	4.00
Ovine	5.00	NA
Caprine	5.00	4.00
Cervine	4.00	2.00
Porcine	4.00	2.00
Ratite	4.00	2.00

3.27.2 M-alerts

(1) An M-alert is determined from both a single processing week's results and a moving window of 5 sets of samples.

(2) The results from the newest set of samples displace the oldest set of results in a moving window.

(3) An M-alert for a product type is generated if:

a) 2 or more samples in a processing week exceed the M-limit; or
b) 3 or more samples above the M-limit are recorded within a moving window of 5 sets of samples.

(4) Any missed samples count as exceeding the M-limit.

(5) Any APC or *E. coli* TNTC results count as exceeding the M-limit.

(6) The moving window is reset after an M-alert is raised.

Guidance

- If the moving window M-alert is breached, this will be expressed automatically in each premises' NMD M-alert ledger. For standard throughput premises a moving window will be 5 weeks. For VLT premises a moving window will be 5 months.
- Product types include carcasses, cuts and bulk as applicable to the species. If multiple carcass sites are sampled, results from all sites are combined in a single moving window. For example, a bovine operator would have moving windows for carcasses, primal cuts, and bulk (i.e. 3 moving windows).

3.27.3 M-alert responses

(1) On the first M-alert from the start of processing under this notice, or after 5 weeks or more of processing without an M-alert, when an M-alert occurs, an operator must:

a) within 1 working day, begin a review of the process to identify and document factors that may have compromised hygienic processing; and
b) take corrective and preventative action if factors that have compromised hygienic processing have been identified.

(2) If another M-alert is generated in the next 5 processing weeks, the operator must also:

a) within 1 working day, notify the premises verifier that an M-alert was triggered and the premises verifier must be involved in the review;
b) within 1 working day, begin a review of the process to identify and document factors that may have compromised hygienic processing; and
c) take corrective and preventative action if factors that have compromised hygienic processing have been identified.

Guidance as to MPI's response

MPI may intervene directly in the process. This may involve:

- products dispositions (e.g. market restrictions, downgrading);
- slowing the chain speed, or stopping the chain; or
- any other action that is considered appropriate by MPI, such as enhanced regulatory oversight.

3.28 SPS for bovine, caprine, ratite, and bobby calf

3.28.1 Detection of *Salmonella*

(1) When notified by a laboratory of any detection of *Salmonella*, the operator must:

a) determine if any personnel handling either animals or product have any symptoms of salmonellosis;
b) consult any laboratory providing an animal health service in the stock catchment area as to any increase in reported cases of animal salmonellosis; and
c) undertake the process responses specified in clause 3.28.2, which escalate according to the frequency of *Salmonella* detections in the season.

3.28.2 Process response to detections

(1) After being notified by the laboratory of any detection of *Salmonella*, the operator must commence a new SW in the next sampling week or, if the detection occurs within the PSW, reset the PSW.

(2) On the first detection of *Salmonella*, the operator must:

a) within 1 working day, begin a review of the process to identify and document factors that may have compromised hygienic processing; and
b) take corrective and preventative action if factors that have compromised hygienic processing have been identified.

(3) On the second or any subsequent detection of *Salmonella* in the moving 6 consecutive processing weeks window following a prior detection, the operator must:

a) within 1 working day, notify the premises verifier that *Salmonella* was detected;
b) within 1 working day, begin a review of the process to identify and document factors that may have compromised hygienic processing;
c) take corrective and preventative action if factors that have compromised hygienic processing have been identified; and
d) take an escalating response if repeat detections occur.

Guidance as to MPI's response

If repetitive detections occur, MPI may intervene directly in the process. This may involve:

- products dispositions (e.g. market restrictions, downgrading)
- slowing the chain speed, or stopping the chain; or
- any other action that is considered appropriate by MPI, such as enhanced regulatory oversight.

Part 4: NMD Poultry Programme

4.1 Application

(1) This Part applies to:

a) an operator required to take samples for the purpose of NMD testing of poultry; and
b) a laboratory engaged by an operator to undertake the required NMD tests.

4.2 Interpretation

(1) Additional terms this Part, unless the context otherwise requires:

breeder chicken means a parent or grandparent bird (male or female) from a poultry farm that produces fertile eggs for the broiler poultry hatcheries for chick production or breeder laying operations

EOL means an end-of-lay chicken, including a spent layer hen or spent breeder chicken

farm means the location at which the poultry sheds are situated

mCCDA means modified charcoal cefoperazone deoxycholate agar, the plating media used for the *Campylobacter* direct plate enumeration method

meat chicken means any of the following:

a) a broiler chicken (ie, a chicken produced primarily for meat);
b) a small breed chicken (ie, a breed of small chickens (such as a Silkie or Silky), and start-of-lay pullets);
c) a poussin (ie, a chicken less than 28 days old at slaughter and weighing no more than 750g, or any other young chicken weighing no more than 850g (a spring chicken).

new start-up premises means a premises processing meat chickens for the first time under a newly registered RMP until the premises achieves a clear CPT moving window

primary chilling means the initial chilling of a poultry carcass by immersion chilling or air chilling

processing day means a day when the primary processing of poultry occurs

shed means a shelter for poultry with facilities for the delivery of feed and water, and may define a poultry flock for traceability purposes

ssBPW means single strength buffered peptone water

whole flock health procedures means, in relation to a flock of farmed poultry, the procedures required by clause G1.6 of the Animal Products Notice: Production, Supply and Processing (which include measures to reduce infection of flocks by *Campylobacter* or *Salmonella*).

4.3 Poultry to be sampled

(1) The NMD Poultry Programme monitors *Salmonella* and *Campylobacter* contamination of poultry through 5 independent sampling programmes as follows:

a) ducks;
b) EOLs;
c) meat chickens;
d) turkeys; and
e) new start-up premises.

4.3.1 Standard throughput premises

(1) A standard throughput premises operator must ensure that 3 carcasses of each type of poultry that is processed in a processing day are sampled for NMD testing.

4.3.2 VLT premises

(1) A VLT premises operator must ensure that 3 carcasses are sampled for NMD testing each processing week and, if more than one poultry type is processed:

a) all 3 carcasses must come from the same poultry type;
b) the type of poultry must be randomly selected; and
c) all 3 carcasses must come from the same processing day.

(2) However, for a new start-up VLT premises that processes meat chickens, 3 meat chicken carcasses must be sampled for NMD testing each processing day until a clear moving window for CPT is achieved (in addition to the 3 carcasses of any other poultry types, as required by subclause (1)).

4.4 Tests required for poultry samples

(1) Operators must ensure all samples are tested for *Campylobacter*.

(2) For standard throughput premises, one sample from the three samples taken on a processing day must be tested for the presence of *Salmonella* (in addition to being tested for *Campylobacter* under subclause (1)).

(3) For VLT premises, one sample from the three samples taken in a processing week must be tested for the presence of *Salmonella* (in addition to being tested for *Campylobacter* under subclause (1)).

4.5 Sample selection

4.5.1 Sample selection plan

(1) Every operator must have a sample selection plan that implements the requirements of this clause in relation to each type of poultry processed.

(2) The operator must keep records relating to sample selection criteria.

4.5.2 Standard throughput premises: Random selection of sampling times

(1) Operators of standard throughput premises must ensure that, for each product type to be sampled, 3 discrete sample times are randomly selected prior to processing based on:

a) 3 unique carcass identification numbers across the entire processing day; or
b) 3 discrete times (in minutes) that processing is reasonably likely to be underway that processing day.

(2) The randomly selected times (reported in minutes) must be recorded prior to sampling.

4.5.3 VLT premises: Random selection of sampling day, time and carcasses

(1) Operators of VLT premises must ensure that all 3 carcasses are selected from one randomly selected processing day, at a randomly selected time, during any processing week as follows:

a) if processing occurs on only 1 processing day of the processing week, then this is the sampling day;
b) if processing occurs on more than 1 day in any processing week, then the sampling day must be randomly selected from the known processing days; or
c) if there is doubt about when processing will occur, samples must be taken on the first processing day of that processing week.

(2) The operator must ensure that the initial carcass is randomly selected from all times that processing is reasonably likely to occur on that processing day as per clause 4.3.2.

(3) The remaining 2 carcasses must be collected at time intervals equivalent to the time required to bag or rinse each carcass.

4.5.4 Departure from the original sample selection

(1) If a problem occurs during the collection of a sample, the operator must ensure that sample is discarded and the next available carcass must be selected for sampling.

(2) If a sample is missed, or if the processing schedules change in a manner that means that the original random sampling plan cannot be followed, the operator must ensure that every reasonable effort is made to randomly sample carcasses from within the remaining processing time available to meet requirements under this notice.

(3) The operator must ensure that any variation to the random selection of sampling days or times is recorded in the operator's random sample selection records.

(4) If the required samples are not collected, each missed sample will be considered a "missed sample".

4.6 Sampling equipment

(1) Operators must ensure that sample-takers have appropriate sampling gear and equipment ready for undertaking whole carcass rinse sampling.

Guidance

Sampling equipment should include:

a) plastic sample bags with twist ties or equivalent, such as poultry rinse bags with a tear tie;
b) insulated container and pre-cooled sterile BPW diluent;
c) a means to record sampling details and label vials;
d) the appropriate number of vials to collect a 70ml sample of whole carcass rinse sample if this occurs in the processing area, and a vial containing liquid to verify temperature of the sample on receipt by the laboratory;
e) Ethanol/iso-propanol sanitising wipes; and
f) a means to time the 2 minute carcass rinse procedure.

4.7 Diluents for sampling and testing

4.7.1 Diluents

(1) Operators must ensure that:

a) the laboratory provides sample-takers with:

i) sterile diluents that are correctly prepared as per table 14 and labelled; and
ii) sterile diluents used for sampling and testing that are not cloudy, loose lidded, odorous, or have passed their expiry dates; and

b) sample-takers use the appropriate diluent according to whether antibacterial agents were applied to the carcass during processing.

4.7.2 Single strength buffered peptone water (ssBPW)

(1) The laboratory must ensure ssBPW used for carcass rinse sampling for *Campylobacter* and *Salmonella* testing is prepared with the diluent in Table 14 and be sterilised.

Table 14

ssBPW Diluent	Amount
Peptone	10.0g
Sodium chloride (NaCl)	5.0g
Disodium phosphate (Na_2HPO_4)	3.5g
Potassium phosphate (KH_2PO_4)	1.5g
Distilled water	1000ml
Final pH 7.2±0.2 at 25ºC	

(2) The operator must ensure that the appropriate volumes of sterile ssBPW diluent are used for sampling of the poultry carcasses using the whole carcass rinse procedure:

a) 400ml of ssBPW per carcass is required for rinses of meat chickens, ducks, and spent layer hen EOLs; and
b) 600ml of ssBPW per carcass is required for rinses of turkeys and breeder chicken EOLs.

(3) The laboratory must use the appropriate volumes of sterile ssBPW diluent for performing the laboratory analysis for *Campylobacter* when undertaking a 10-fold dilution series on whole carcass rinsate samples.

4.7.3 Antibacterial agents

(1) Prior to sampling, the operator must inform the laboratory if antibacterial agents are used during processing.

(2) If antibacterial agents are used during processing, the laboratory must prepare the appropriate ssBPW diluent for sampling (not testing) by:

a) determining suitable non-antimicrobial neutralising additives;
b) adding these to the diluent if required; and
c) labelling the mixture accordingly.

Guidance

- For example, if a chlorinated compound, such as acidified sodium chlorite (ASC) has been used during processing, the laboratory must ensure that a peptone diluent with the addition of 1.0ml of a 3% sodium thiosulphate ($Na_2S_2O_3$) solution to 1 litre of diluent is used for sampling.

4.8 Sample collection

(1) Operators must ensure that samples are collected in accordance with this clause.

4.8.1 Poultry carcass selection criteria

(1) A whole poultry carcass must be selected for sampling from the line after primary chilling at the last readily accessible point prior to the carcass entering any packing bins, being bagged, cut up or sent for further processing.

(2) If the carcasses are mixed in bins at manual grading, the carcasses must be selected immediately prior to grading (for example, off the drain-table, after draining).

(3) If the carcasses are re-hung on the chain after primary chilling, the carcass must be selected immediately prior to the first drop point, or similar, at which the carcasses enter secondary processing or are bagged for retail.

(4) If the position of the first drop point is not readily accessible, the operator must:

a) determine the time the carcass would normally take to move from the point it will be selected from the line to the first drop point (known as T^0); and
b) communicate this information to the laboratory and the sample-taker.

4.8.2 Taking samples

(1) The carcasses must be sampled no more than 10 minutes either side of the originally selected random sampling time.

(2) If the carcass is selected prior to the first drop point, the selected carcass must be hung for time T^0 determined in subclause 4.8.1 (4). If the carcass is selected directly from grading bins, this is not required.

(3) The sampling time must be recorded using a format that can be translated to 24-hour clock units when the sample description information and results are entered into the NMD database:

a) for standard throughput premises, record the time for each of the 3 poultry carcasses selected during that processing day; or
b) for VLT premises, record the time for the first carcass selected.

(4) Carcasses must be handled aseptically during sampling and the carcass rinse procedure.

(5) Each carcass must be placed in a sterile sampling bag.

(6) Any whole carcass rinse must be undertaken according to the procedure in clause 4.8.3:

a) in the processing area of the poultry premises; or
b) in a laboratory.

(7) Carcasses for the day must not be collected all at once from the line and then bagged or rinsed unless there are no other opportunities to complete collection of the 3 carcasses.

4.8.3 Carcass rinse procedure

(1) The carcass rinse procedure must be performed as follows:

a) pour the required volume of the chilled sterile ssBPW diluent over the carcass within the bag. Expel most of the air from the bag and then secure the bag tightly by hand or with a twist tie to prevent losing any of the diluent;
b) commence the whole carcass rinse procedure and begin timing. This must continue for 2 minutes to ensure complete coverage of both the external and internal surfaces of the carcass with the diluent, which becomes the 'rinsate' when the rinse procedure begins;
c) rinse the carcass: begin by rocking the carcass in the bag in an arching motion to rinse the outside of the carcass, including the wings and legs. Transfer the weight of the carcass from one hand to the other while simultaneously massaging the carcass surface, particularly around the wings and the legs. When the rinsate becomes yellowy in colour (with suspended fats), proceed to the next step;
d) ensure that the cavity of the carcass is rinsed by periodically positioning the carcass so that the rinsate can enter. Any fat or skin covering the vent may need to be cleared to enable the rinsate to enter the cavity;
e) swirl the rinsate so that the entire internal cavity is rinsed and the rinsate on exiting from the cavity is a pink to red colour; and
f) continue rocking the carcass to pass the rinsate over the external and internal surfaces for the remainder of the 2 minutes. There should be at least 2 complete internal cavity rinses during the 2 minutes. On completion of the whole carcass rinse, check that the rinsate has a reddish tint and is thicker than the starting diluent due to the presence of suspended fats and visible cells.

4.8.4 Collecting the carcass rinsate

(1) Collect the carcass rinsate by either of these 2 methods:

a) whole sample bag of rinsate:

i) place the sample bag and carcass on a flat surface;
ii) open the bag and remove the carcass aseptically using a gloved hand; and
iii) expel the air from the sample bag (for ease of packaging) and secure the top to prevent contamination and/or spilling of the rinse sample; or

b) collection of an aliquot of rinsate:

i) before removing the carcass aseptically transfer at least 70ml of the rinsate to a sterile vial.

4.8.5 Carcasses after rinsing

(1) If carcasses that have been rinsed in the processing area of the poultry premises are intended for human consumption, they must be returned to a point in the chain where subsequent processing will ensure that the carcass meets requirements for human consumption.

(2) Carcasses sent to the laboratory must not be supplied by any person for human consumption.

4.9 Sample description information

(1) Operators must ensure that the sample description information in subclause (2) is:

a) recorded directly into the NMD database prior to transport of the sample to the laboratory; or
b) by prior arrangement with the laboratory, recorded on a sample submission form sent to the laboratory with the samples.

(2) The sample description information required is:

a) the name of the sample-taker:
b) the poultry premises from which samples were taken:
c) the following specific information associated with the poultry provided to the premises:

i) the number of times a shed has been entered to partially or fully depopulate it (ie, the cut number);
ii) the average age of birds;
iii) the farm reference number and shed number;
iv) whether the poultry is free range or not free range;
v) poultry type (ie, meat chicken, EOL, duck, or turkey);

d) class of meat chicken (broiler, poussin, or small breeds);
e) class of EOLs (breeder or layer);
f) sampling date;
g) the time (24-hour clock) the sample was taken;
h) the method of drainage and, if required, the drainage time; and
i) the location where the carcass rinse procedure was conducted (poultry premises or laboratory).

4.10 Storing and transporting samples to the laboratory

(1) Operators must ensure that samples are prepared and transported to the laboratory in accordance with this clause.

4.10.1 Preparing samples for transport

(1) The following must be tightly sealed for transport to ensure the contents are secure:

a) the sample bag containing the carcass rinsate, with or without the poultry carcass;
b) the aseptically collected rinsate in a sterile vial; and
c) the unrinsed carcass in the sample bag.

(2) All secured samples must be immediately placed into the pre-cooled insulated container.

(3) The samples must not be in direct contact with any frozen coolant used.

(4) Samples awaiting transport to the laboratory must be stored in a refrigerator operating in the calibrated temperature range of 0°C to 5°C.

4.10.2 Delivery of samples to the laboratory

(1) Whole carcasses transported to the laboratory for rinsing must be:

a) transported in insulated containers with sufficient frozen coolant to cool the sample;
b) delivered to the laboratory no later than 30 minutes after selection of the whole carcass from the processing chain; and
c) rinsed immediately upon receipt by the laboratory and no later than 60 minutes after the carcass was selected from the line.

(2) Carcass rinse samples must be transported to the laboratory so that:

a) the temperature of the rinsate is maintained at a temperature between 0°C and 5°C; and
b) laboratory testing can be initiated within 30 hours from when the first sample was collected.

4.11 Receipt of samples by laboratory

(1) A laboratory that receives samples must:

a) record in the sample register the sample temperature on receipt (for carcass rinse samples);
b) reject samples that are less than 0°C, or samples that exceed 10°C;
c) confirm that the samples are suitable for testing;
d) confirm that testing will be initiated within 30 hours from time of collection of the first sample;
e) confirm that the person recorded as collecting the samples is a sample-taker whose details are held by the laboratory; and
f) enter the sample description information supplied by the sample-taker on the submission form into the NMD database if not already entered by the sample-taker.

(2) If samples submitted by the operator are not suitable for testing, the laboratory must reject the samples, and seek replacement samples from the same processing day (standard throughput premises) or processing week (VLT premises).

(3) The laboratory must record the rejected samples as a technical failure, with reasons, in the NMD database.

4.12 Laboratory testing

(1) Following receipt of samples, a laboratory must:

a) if the samples received are whole carcasses, rinse the carcasses as required by clauses 4.8.3 - 4.8.5;
b) test each rinse sample for *Campylobacter*; and
c) randomly designate a rinse sample from the 3 carcasses and test it for *Salmonella* (in addition to the *Campylobacter* testing*)*.

4.13 *Campylobacter* testing

(1) Every laboratory must undertake *Campylobacter* testing for the purposes of this Notice in accordance with this clause.

4.13.1 Preparing for testing

4.13.1.1 Dilutions required

(1) A 10-fold serial dilution series must be used for the analysis of *Campylobacter*.

(2) The dilutions 10^0 and 10^1 are required in addition to sufficient higher dilutions to make sure that the maximum allowable count is obtained on at least one, or one set of, plates. (The undiluted carcass rinsate is the 10^0 dilution for the purposes of calculations.)

(3) The laboratory must ensure that only plates with counts that fall in the acceptable counting range as in Table 15 are counted:

Table 15

Plate dilution	Allowable count for mCCDA direct plate *Campylobacter*
10^0 dilution	0 - 150 CFU
All other dilutions	15 - 150 CFU

4.13.1.2 High end dilutions and TNTC results

(1) If a count exceeds the maximum allowable count on the highest dilution (above), a TNTC result must be recorded.

(2) The test does not need to be repeated for the sample that has a TNTC result.

(3) If a TNTC result occurs, the laboratory must extend the dilution series, to ensure results are within the acceptable counting range for subsequent samples submitted by the premises in question.

4.13.1.3 Preparing dilution volumes

(1) All serial dilutions for whole poultry carcass rinse samples must be performed using ssBPW and either of the 2 dilution volumes:

a) 9ml in 25ml universal bottles or vials, with 1ml transfer volume; or
b) 4.5ml in microcentrifuge or microdilution tubes, with 0.5ml transfer volume.

(2) The use of pre-poured dilution volume is allowed only if a laboratory can provide evidence that:

a) they do not lose volume during autoclaving; and
b) they have an appropriate quality assurance programme in place for ongoing verification.

(3) A fresh pipette or pipette tip must be used for each transfer of diluted sample.

(4) The bacteria in the dilutions must be as homogenously distributed as possible at all stages.

4.13.2 *Campylobacter* Direct Plate enumeration method

(1) The *Campylobacter* Direct Plate enumeration method outlined in this clause must be used when undertaking testing for *Campylobacter* for poultry carcass rinsate samples under this notice.

4.13.3 Preparing mCCDA plating media

(1) The plating media mCCDA must be used for *Campylobacter* testing.

(2) Dehydrated mCCDA basal medium (commercially available) must contain the proportions of ingredients in Table 16.

Table 16

Basal medium components	Quantity in grams (g) per 1000ml
Lab-lemco powder	10.0
Peptone	10.0
Sodium chloride	5.0
Bacteriological charcoal	4.0
Casein hydrolysate	3.0
Sodium deoxycholate	1.0
Ferrous sulphate	0.25
Sodium pyruvate	0.25
Agar	12.0

(3) Suspend 22.75g of dehydrated mCCDA basal medium in 500ml of distilled water, mix well and boil to dissolve the agar.

(4) Sterilise by autoclaving at 121°C for 15 minutes.

(5) The final pH of the mCCDA basal medium must be pH 7.4±0.2.

(6) To complete the medium, add mCCDA Selective Supplement (commercially available) containing Cefoperazone 16 mg and Amphotericin B 5 mg to the mCCDA basal medium as follows:

a) cool the autoclaved mCCDA basal medium to about 50°C; and
b) aseptically add each selective supplement to 500ml of the mCCDA base; and
c) mix thoroughly and pour into Petri plates.

(7) mCCDA plates can be stored for up to two weeks in sealed containers at 4°C.

(8) The mCCDA plates must be air dried before use, either by leaving unopened on the bench overnight, or when plates are used on the day of preparation, in an incubator at 42°C.

(9) The mCCDA plates must not be dried in a laminar flow cabinet.

4.13.3.1 Initiation of analysis

(1) Initiation of analysis for *Campylobacter* commences when the rinsate is dispersed across the plates.

4.13.3.2 Direct plating

(1) Carry out all quality control procedures for the media and methods using *Campylobacter jejuni* NZRM 1958 as a positive control and *Escherichia coli* NZRM 916 as a negative control.

(2) Take 2ml of rinsate (10^0 dilution) from the sample bag or sample vial and apply over 6 dried and labelled mCCDA plates. All of the 2ml must be dispensed over the 6 plates using approximately equal volumes. The exact volume dispensed per plate does not need to be recorded.

(3) Spread each individual volume dispensed on the agar surface with the same sterile spreader. Allow the 6 plates to remain upright at room temperature to permit the sample to soak in before inverting.

(4) Also plate 2 further volumes of 0.1ml of the rinsate (10^0 dilution) onto 2 additional mCCDA plates.

(5) If a higher concentration of *Campylobacter* is expected, undertake additional 10 fold serial dilution using sterile ssBPW. Plate 0.1ml of each dilution (in duplicate) onto mCCDA plates.

(6) Incubate all of the plates for 48 hours ± 2 hours at a temperature of 42± 0.5ºC in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). Maintain the microaerobic atmosphere at all stages of incubation.

Guidance

- An appropriate number of sachets for the jar/container should be used. If too many sachets are used a moist environment and condensate on plates will occur, which could influence colony growth and cause colony spreading. Too many or too few sachets will not create the correct gaseous mixture in the container.

4.13.3.3 Presumptive count

(1) Select a set of plates with not more than 150 colonies per plate from any serial dilution. These may be any of the dilutions, from either the 2ml set of 6 plates, or from the 0.1ml duplicate plates. Plates with counts of over 150 are considered TNTC.

(2) Examine these plates for typical thermotolerant *Campylobacter* spp. colonies: flat grey and moistened, variable size for 1mm - 5mm in diameter, from pinpoint colonies to roundish or flattened out colonies with irregular spreading margins.

(3) Count and record the numbers of typical thermotolerant *Campylobacter* spp. colonies on this set of plates for the dilution.

Guidance

- Other organisms that may be present on mCCDA plates include creamy coloured yeast colonies, which can easily be distinguished by 10x magnification, and *Arcobacter* and Pseudomonads.

4.13.3.4 *Campylobacter* confirmation

(1) *Campylobacter* confirmation must be conducted on fresh colonies from mCCDA agar plates immediately following incubation and the presumptive count to confirm the presence of thermotolerant *Campylobacter* spp. Speciation of the *Campylobacter* is not required.

(2) Select 5 typical colonies, or all colonies if there are fewer than 5 colonies. Screen all 5 colonies for oxidase activity. Record the result.

Guidance

- Small colonies may not contain enough cells to perform the oxidase and confirmation tests. To ensure that there are sufficient cells available, small colonies should be subcultured to blood agar prior to confirmation.

(3) On the first oxidase positive colony carry out confirmation using a latex agglutination test or other validated method that the laboratory has been accredited for to confirm for *Campylobacter*, and:

a) if the confirmation test is positive, assume that all of the colonies are *Campylobacter* positive; or
b) if not, conduct the confirmation test on the remaining oxidase positive colonies.

(4) If there are no typical colonies present, no further confirmation is required.

(5) Record the colony count and associated dilution for purposes of calculating the number of *Campylobacter*. To enumerate *Campylobacter* count:

a) the total number of colonies that were selected for oxidase testing = n; and
b) the number of those colonies that were confirmed (or assumed) positive for thermotolerant *Campylobacter* spp.

Guidance **Example of positive results by oxidase and confirmation testing**			
Typical colonies selected	**Oxidase**	**Confirmation test**	**Result per colony**
1	positive	positive	*Campylobacter*
2	positive	presume remaining oxidase positive are *Campylobacter* positive	*Campylobacter*
3	positive		*Campylobacter*
4	positive		*Campylobacter*
5	positive		*Campylobacter*
Example of negative results if the first oxidase colony of the selected colonies records a negative confirmation result.			
Typical colonies selected	**Oxidase**	**Confirmation test**	**Result per colony**
1	negative	not conducted	negative
2	positive	negative	negative
3	positive	positive	*Campylobacter*
4	positive	positive	*Campylobacter*
5	negative	not conducted	negative

4.13.3.5 Reporting *Campylobacter* results (*Campylobacter* enumeration)

(1) If there are no typical *Campylobacter* colonies present on the mCCDA plates following the incubation, then report the results as "*Campylobacter*: not detected".

(2) If the number of colonies exceeds 150 on any of the mCCDA plates with the highest dilution in the sample set, report the result as "TNTC".

(3) Record presumptive *Campylobacter* spp. colonies as follows:

a) if no thermotolerant *Campylobacter spp.* colonies were confirmed, report as "not detected"; or
b) if thermotolerant *Campylobacter spp.* colonies were confirmed, calculations to determine the number of colony forming units (CFU) per carcass need to be undertaken as per clause 4.15.

4.13.3.6 Weekly check of confirmation method

(1) The laboratory must check the method of *Campylobacter* confirmation once a week as follows:

a) choose a set of 5 characteristic *Campylobacter* colonies from mCCDA agar plates that are oxidase positive; and
b) test all 5 with the confirmation test to validate the presumption that an oxidase positive test will be *Campylobacter* positive.

4.14 *Salmonella* testing

(1) Every laboratory must undertake detection for *Salmonella* in poultry in accordance with this clause.

(2) All quality control procedures for the media and methods must be carried out using:

a) *Salmonella Menston* NZRM 383 as the positive control; and
b) *E. coli* NZRM 916 as the negative control.

4.14.1 Initiation of analysis

(1) Initiation of *Salmonella* analysis for *Salmonella* commences when the ssBPW suspension is placed in the incubator.

4.14.2 Carcass rinse sample for *Salmonella* testing

(1) Samples are derived from the whole poultry carcass rinsate.

(2) Randomly select 1 of the 3 samples for *Salmonella* testing.

4.14.3 Pre-enrichment step

(1) Transfer 30ml of the carcass rinsate to a separate sterile vial for use as the ssBPW suspension used for the pre-enrichment.

(2) Immediately after preparing the ssBPW suspension, incubate the pre-enrichment samples at 35±1ºC or 37±1ºC for 18-24 hours.

(3) Once the *Salmonella* analysis has been initiated, all steps must be completed within the time and temperature requirements and the ssBPW suspension and/or the Rappaport-Vassiliadis Salmonella (RVS) selective enrichment broth must not be refrigerated at any time.

4.14.4 Enrichment

(1) Following incubation transfer 0.1ml of the ssBPW enrichment culture to 10ml of RVS selective enrichment broth pre-warmed to 42°C.

(2) Incubate the RVS selective enrichment broth for 24 hours ± 2 hours at 42±0.2°C in a water bath or incubator.

4.14.5 Plating

(1) Following incubation transfer a loopful (10µl) of the RVS enrichment culture to one plate of Brilliant Green Modified (BGM) agar and to a plate of Xylose Lysine Desoxycholate (XLD) agar selective plating media and streak to obtain single, well isolated colonies. Label plates with the agar type (BGM or XLD) as the agar colour can change during incubation making it hard to distinguish between the 2 types of media.

(2) Incubate both agar plates for 18-24 hours at 35±1°C or 37±1°C.

(3) Examine the agar plates for the presence of typical *Salmonella* colonies:

a) BGM agar: pink colonies surrounded by bright red medium; and
b) XLD agar: red colonies with a black centre (H_2S negative serotypes have red colonies without a black centre).

4.14.6 Confirming presumptive positive *Salmonella*

(1) Select 5 or more single typical *Salmonella* colonies from the XLD and/or BGM selective plating media for confirmation purposes.

(2) Use biochemical tests and/or commercial kits as listed in MIMM Identification of *Salmonella* Colonies and perform the agglutination reaction as per the manufacturer's instructions, testing each of the 5 colonies sequentially.

(3) If any one of these colonies is positive the sample is deemed positive.

(4) Upon detection of a positive colony, any remaining colonies need not be tested.

4.14.7 Final confirmation and serological typing

(1) Applies when testing, as required in 4.2.2, for *Salmonella* in any duck, EOLs, meat chicken or turkey carcass, returns a positive *Salmonella*, as in 4.11.6

(2) Streak a positive colony from either selective media onto MacConkey agar (without salt and crystal violet).

(3) Incubate for 24h at 35±1ºC or 37±1ºC.

(4) Subculture any typical colourless colonies onto plate count agar and incubate overnight at 35±1ºC or 37±1ºC.

(5) Following overnight incubation, colonies must be submitted to the Institute of Environmental Science and Research (ESR) Enteric and *Leptospira* Reference Laboratory, Wallaceville directly.

4.14.8 Recording *Salmonella* results

(1) For poultry whole carcass rinse samples, record the *Salmonella* results as "not detected" or "detected".

4.15 Reporting results

4.15.1 Reporting in NMD database

(1) Laboratories must enter the colony counts from *Campylobacter* plates, and the presence/absence results in the case of *Salmonella*, in the NMD database.

Guidance

- The *Salmonella* serotype and the Enteric Reference Laboratory (ERL) report number, confirmed result and serotyping for *Salmonella* are entered by ESR on confirmation that the report has been signed an ESR analyst qualified for *Salmonella*.

4.15.2 Manual calculation of results

(1) If required for the purpose of reporting to operators, laboratories must use manual calculation to determine final results, in accordance with subclauses (2) to (4).

(2) Calculation requires the counts of the total number of presumptive *Campylobacter* spp. colonies on the set of plates, the number of colonies with the characteristic *Campylobacter* morphology selected for confirmation (n), and the number of colonies confirmed as being a thermotolerant *Campylobacter.*

(3) For counts obtained from 2ml of the 10^0 dilution spread over 6 plates the formula is:

a) CFU/carcass = (number of colonies confirmed as *Campylobacter* spp./n) x total count of typical *Campylobacter* morphology colonies (plate 1 + plate 2 + plate 3 + plate 4 + plate 5 + plate 6) x rinsate volume (400ml or 600ml)/2ml = number of *Campylobacter* organisms/poultry carcass sample.

(4) For counts obtained from duplicate plates of higher dilutions the formula is:

a) CFU/carcass = (number of colonies confirmed as *Campylobacter* spp./n) x count of typical *Campylobacter* morphology colonies (plate 1 + plate 2)/2 x rinsate volume (400ml or 600ml)/0.1ml x 1/dilution = number of *Campylobacter* organisms/poultry carcass sample.

Guidance

- The ratio of the '(number of colonies confirmed as *Campylobacter* spp./n)' is usually 5 out of 5 or will be a proportion of 5 if the remaining colonies had to be confirmed, e.g. 3/5.
- If fewer than 5 typical colonies were available for oxidase and confirmation tests, then the ratio will be the number of colonies confirmed as *Campylobacter* spp. divided by the number of typical colonies selected, e.g. 1/3.

4.15.3 Limits of detection

(1) The laboratory must ensure results are entered in accordance with this clause.

(2) "Not detected" are considered to be results less than the limit of detection.

(3) The limit of detection for NMD *Campylobacter* enumeration is where only a single colony on 1 of the 6 zero dilution plates is confirmed as *Campylobacter* spp. such that the *Campylobacter* spp. count of 5 plates of 6 = 0, and the count on only 1 plate of the 6 = 1.

(4) The values used to represent the limit of detection and "not detected" results in the NMD database are as set out in Table 17:

Table 17

Test	**Lowest Limit of Detection Value**		**"Not Detected"**
Campylobacter direct plating	CFU/carcass	Log_{10} value per carcass	Log_{10} value per carcass
2ml for 400ml	200	2.30	2.00
2ml for 600ml	300	2.48	2.00

(5) A "not detected" *Campylobacter* result will be recorded as 2.00 log_{10}CFU/carcass in the NMD database.

4.15.4 Recording sample description information and default results

(1) The laboratory must ensure sample description information is recorded in the NMD database for every sample taken.

(2) If sample description information is not entered, a missed sample result will be recorded in the NMD database for that processing day or processing week.

4.15.4.1 Missed samples

(1) Missed samples occur when fewer samples are collected than are required to make a complete set of samples.

(2) Each missed sample will default to a *Campylobacter* result of greater than 3.78 log_{10} CFU/carcass result recorded as 3.79 log_{10} CFU/carcass result on the NMD database.

4.15.4.2 Technical failures

(1) If samples have been collected but a technical failure (TF) has not permitted a result, the sample description information must be entered as proof of sampling with TF recorded in the result field. Entering the sample description information ensures that a *Campylobacter* 3.79 log_{10} CFU/carcass default result is not generated.

4.15.4.3 Too numerous to count *Campylobacter* results

(1) TNTC results occur when counts are greater than the allowable counts on any of the plates in the highest dilution plated as specified in Table 15.

(2) TNTC results must be reported.

(3) Each TNTC result will default to a greater than the 3.78 log_{10} CFU/carcass result; recorded as 3.79 log_{10} CFU/carcass on the NMD database.

4.16 SPS for meat chickens

(1) The regulatory limit for *Salmonella* detections in meat chickens is detection in no more than 5 carcass rinse samples in a 51 sample moving window.

4.16.1 Detection of *Salmonella*

(1) When notified by a laboratory that *Salmonella* has been detected the operator must:

a) immediately inform the verifier of the detection;
b) ensure the laboratory has submitted purified cultures of isolates detected to the Institute of Environmental Science and Research Limited (ESR) Enteric and Leptospira Reference Laboratory, Wallaceville, for serotype identification;
c) ensure records from the original product sampled are traced back to the catchment area of the chicken being processed and review the whole flock health procedures;
d) determine if any personnel handling either the birds or the carcasses have any symptoms of salmonellosis; and
e) consult any laboratory providing an animal health service in the stock catchment area, and inquire as to any increase in reported cases of animal and poultry salmonellosis.

4.16.2 Process responses

(1) On breaching the SPS, the operator must:

a) immediately review the process, chicken flocks and feed *Salmonella* status to identify and document factors that resulted in breach of the SPS;
b) review and consider whether the process control programmes (pre-requisite and HACCP) must be modified;
c) provide the verifier with a *Salmonella* Management Plan within 7 days of, describing:

i) the process/HACCP reviews; and
ii) measures implemented to reduce the prevalence of pathogens.

Guidance as to MPI's response

- Following a breach of the SPS, the Director-General may intervene directly in an operator's operations, including by such things as:
  - increasing the frequency of verification;
  - requiring full-time supervision of processing;
  - introducing further interventions;
  - specifying product disposition;
  - requiring further sampling and research initiatives;
  - requiring premises closure.

4.17 CPT for meat chickens

(1) The CPT for meat chickens has the following two regulatory limits:

a) an enumeration target, which is:

i) for standard throughput premises, that no more than 2 individual carcass rinse samples taken from one moving window may have *Campylobacter* counts greater than 6000 CFU per carcass (3.78 $\log_{10}$CFU/carcass); and
ii) for VLT premises, that no more than 1 individual carcass rinse sample taken from one moving window may have *Campylobacter* counts greater than 6000 CFU per carcass (3.78 $\log_{10}$CFU/carcass):

b) a detection target, which is:

i) for standard throughput premises, that no more than 10 individual carcass samples taken from one moving window may have *Campylobacter* counts of 2.30 $\log_{10}$ CFU/carcass or greater; and

ii) for VLT premises, that no more than 4 individual carcass samples taken from one moving window may have *Campylobacter* counts of 2.30 $\log_{10}$ CFU/carcass or greater.

(2) The moving window for the purposes of the CPT is 3 successive processing weeks.

(3) The results from the newest set of samples displace the oldest set of results in a moving window.

Guidance

Example CPT sampling requirements (routine processing)

Sampling period	Standard throughput premises	VLT premises
A moving window of 3 processing periods	45 samples over 15 processing days	9 samples over 3 weeks

4.17.1 New start-up premises

(1) An operator of a new start-up premises that is a VLT premises must collect NMD samples each processing day that meat chickens are processed, as required for standard throughput premises operators, until they achieve a clear moving window.

(2) An operator of a new start-up premises that is a VLT premises may move to the usual sampling frequency for VLT premises (see clause 4.3.2) only once a clear moving window for *Campylobacter* is achieved.

Guidance as to MPI's response

- If the *Campylobacter* results remain non-compliant and validation of the process conducted under the new RMP remains in question, the Director-General may impose additional measures and requirements under the Act.

4.17.2 CPT non-compliance

(1) A CPT non-compliance occurs if:

a) the enumeration target is not met, in which case an enumeration failure (EF) is generated; or
b) the detection target is not met, in which case a detection failure (DF) is generated.

(2) A non-compliant moving window occurs when premises are recorded as having an EF, a DF, or both.

(3) To clear a CPT non-compliance and reset to a compliant status, premises must achieve three consecutive clear moving windows (ie, with no EF or DF).

Guidance

- A non-compliance will be recorded in the NMD database as soon as the non-compliance becomes evident (which may be before the results from all samples for that moving window have been entered). This enables corrective actions to be initiated at the earliest opportunity.

4.17.3 Response to CPT non-compliance

(1) As soon as an operator is aware of a CPT non-compliance, the operator must notify their verifier of this within the next 24 hours.

(2) With each non-compliance investigation, corrective actions must be undertaken and any further actions planned to reach a compliant status must be documented by the operator.

(3) In relation to the first and subsequent non-compliances the operator must undertake the actions as set out in Table 18, until achieving 3 consecutive moving windows with no CPT non-compliances.

Table 18

Number of consecutive CPT non-compliances	Responses required to successive CPT non-compliances
1	Within 1 week of a CPT non-compliance, the operator must: (a) investigate and initiate corrective actions to restore control; and (b) report the results to the verifier.
2	Within 1 week of a 2nd consecutive CPT non-compliance, the operator must: (a) document the investigations done, the corrective actions taken to date, and any further actions planned to restore control; and (b) report the results and plans information to the verifier.
3	Within 1 week of a 3rd consecutive CPT non-compliance, the operator must update the documents required after a 2nd non-compliance, and report this to the verifier.
4	Within 1 week of a 4th consecutive CPT non-compliance, the operator must update the documents required after a 2nd non-compliance, and report this to the verifier.
5	Within 1 week of a 5th consecutive CPT non-compliance the operator must update the documents required after a 2nd non-compliance, and report this to the verifier.
6	Within 1 week of a 6th consecutive CPT non-compliance, the operator must: (a) update the documents required after the 2nd non-compliance, and report this to the verifier; and (b) document any product disposition options the operator could implement in order to minimise the risk of contaminated product reaching the consumer, and provide these to the Director-General within a week after notification.
7	Within 1 week of a 7th consecutive CPT non-compliance, the operator must update the documents required after a 2nd non-compliance, and report this to the verifier.
8	Within 1 week of a 8th consecutive CPT non-compliance the operator must update the documents required after a 2nd non-compliance, and report this to the verifier.

Guidance as to MPI's response

- Following a CPT non-compliance, the Director-General may intervene directly in an operator's operations, including by such things as:
  - increasing the frequency of verification;
  - requiring full-time supervision of processing;
  - introducing further interventions;
  - specifying product disposition;
  - requiring further sampling and research initiatives;
  - requiring premises closure.