Meat Standards Committee

Microbiological testing for process monitoring in the meat industry

Guidelines

Approved 30 October, 2002

1 INTRODUCTION

The Australian Standard for the hygienic production and transportation of meat and meat products for human consumption AS 4696-2002, Section 3.12 requires that

"The meat business complies with surveillance (targeted) sampling, monitoring and testing programs(including the National Residue Survey monitoring programs)that:

(a) are endorsed by the relevant Commonwealth, State or Territory authority, and

(b) apply to the meat business."

This Guideline provides protocols for microbiological testing for the purpose of demonstrating process control where required by Controlling Authorities pursuant to Section 3.12 (above) of the Australian Standard.

This Guideline requires:

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

Microbiological testing is carried out by meat processors for a number of reasons including:

- Satisfying regulatory requirements
- Monitoring process control
- Identifying poor performance with a view to improving the process
- Gauging the effectiveness of cleaning procedures
- Providing a customer with information on product quality
- Assessing product against a national or international benchmark

2 SCOPE

This Guideline applies to:

1. Carcases, and raw meat products which are required to comply with the Australian Standard for the hygienic production and transportation of meat and meat products for human consumption AS 4696:2002.
2. Equipment, work surfaces and other surfaces which product and hands may contact incidentally in the course of processing.

3 DEFINITIONS

Cattle Unit	Meat processing throughput measured by equating throughput to cattle as follows: 8 sheep, 6 calves, 6 pigs, 8 goats, 6 deer
Controlling Authority	The Commonwealth, State or Territory authority that is responsible for the enforcement of Australian Standard AS 4696-2002
Corrective Action	Action taken by a meat processor or a regulatory authority in relation to a product or process whenever a microbial count significantly higher than the company baseline is detected
Process Review	A review of processes used in the production of meat to identify causes of high counts and to identify process improvements to reduce those counts
Sampling Plan	A prescribed number of samples for testing to be taken over a given period by a meat processor from product produced at the premises and from work surfaces at that premises
Standard Method	A sampling and/or testing method published in an official reference manual (eg. Standards Australia, Association of Official Analytical Chemists International
Test Methods	Methods prescribed by this Requirement for taking microbiological samples from meat and meat products and testing these samples for the presence of specified microorganisms

4. RESPONSIBILITIES

4.1 Controlling authorities

Controlling Authorities should ensure that sampling and testing programs are undertaken to meet the requirements of this Guideline.

4.2 Meat Processors

Meat processors should:

1. Acquire the necessary equipment and materials to undertake sampling and testing, or arrange for testing to be carried out by a laboratory approved by an organisation recognised by the Controlling Authority
2. Ensure that personnel are competent to perform the testing being undertaken
3. Ensure that sampling and testing is carried out in accordance with these requirements
4. Record testing results to allow monitoring over time
5. Evaluate test results according to broadband microbiological criteria
6. Undertake process review as part of corrective action
7. Make results available to the Controlling Authority or its agents

5 ASSESSING WORK SURFACES

Testing of work surfaces is performed to check the effectiveness of cleaning procedures.

5.1 Sampling and testing

Samples for testing should be collected from cleaned surfaces by testing for TVC at 20-25°C. Testing should be rotated around the plant to provide an overall coverage over time and should be done with sufficient frequency to demonstrate the effectiveness of the cleaning program.

Swabbing and plating, or contact plating are suitable techniques for monitoring the cleaning program.

Petrifilm, Dip Slides or Contact Plates are prepared according to the manufacturer's recommendations and applied to cleaned work surfaces. A proportion of the bacteria adhering to the work surface will be picked up. After incubation, a count can be made of the number of bacteria/cm^2 on the medium reflecting the number on the initial work surface. The count will tell the operator whether the cleandown was Satisfactory (5 colony forming units/cm^2 or less) or Unsatisfactory (more than 5 colony forming units/cm^2).

Other techniques, such as ATP bioluminescence, allow monitoring of cleaning procedures in real time, but have high initial costs compared with the simple methods described above.

It is necessary to use the same method each time testing is performed so that results can be compared and trends in the data can be identified. This should not prevent a processor changing methods from time to time as new methods become available or more suitable techniques are developed.

5.2 Corrective Action

Corrective action for poorly-cleaned surfaces should be developed with particular focus on monitoring the effectiveness of the corrective action. Corrective action can include:

1. Review cleaning program:
   - cleaning method
   - chemicals (used as per manufacturers specifications, and consult chemical supplier)
   - program being followed
   - effectiveness of hygiene monitoring reports
2. Follow up monitoring sufficient to demonstrate that the corrective action was effective

6 ASSESSING MEAT AND MEAT PRODUCTS

Both Total Viable Count (TVC) and *E. coli* testing are necessary to understand the process of slaughter, dressing and chilling. Testing for other organisms may be specified by importing countries or specific customers.

6.1 Sampling and testing

In general, testing is carried out on:

1. Meat surfaces – carcases and primal cuts have bacteria mainly on the surface and these are removed for counting either on a swab/sponge (sponge sampling) or by slicing off a thin layer of meat (excision sampling). The method recommended in this guideline (Appendix D) for routine sampling is for sponge sampling. Beef and pork carcases are sampled by sponging areas of 100 cm^2 (10 cm sides) while smallstock carcases are sampled by sponging areas of 25 cm^2 (5 cm sides).

   Carcase sampling is done after active chilling has cooled and dried the surface

   In the case of hot boning, samples are taken immediately prior to exiting the slaughter floor.

2. Masses – in the case of meat pieces or comminuted meat, the bacteria are distributed throughout the product and bacteria must be removed from the mass. If sampling of meat pieces is required, chilled meat pieces are sampled by coring with a sterile coring tool and frozen meat is sampled with a

sterile drill (diameter 25mm). In each case a sample of 50-100g is taken to the laboratory for testing.

Sampling frequency

Plants with a throughput equivalent to 150 cattle units/week or more should establish a microbiological baseline level by intensive sampling. When plants have demonstrated a satisfactory baseline level, the sampling frequency may be reduced. By contrast, if microbiological levels are not consistently controlled, the sampling frequency should be stepped up until the problem has been solved and a satisfactory baseline established.

Five samples are taken from each production line at each sampling. Where more than one species is dressed on a slaughter chain, each species is separately counted for the purposes of sampling. Testing may be clustered to one or more days of the week providing, over time, samples represent the overall throughput.

Very Small Premises (VSPs), defined as processing the equivalent of <150 cattle units/week, should sample each species routinely processed at the premises at a frequency which demonstrates hygienic processing.

Microbiological methods

Samples are tested using standard methods for Total Viable Count and *E. coli*. Appendix E contains a recommended method but if customers or a Controlling Authority specify different a testing method, this must be followed.

6.2 Corrective action

Corrective action involves focusing on various aspects of the operation eg. contamination levels of livestock, cutting lines through the hide/pelt, specific processes, specific sites on the carcase and chilling practices to determine the origin of high counts. The effectiveness of the corrective action must be documented.

7 MICROBIOLOGICAL SAFETY

Culture plates must always be handled and stored to prevent any bacteria on them coming in contact with people. Although only TVC and *E. coli* are being counted, there is the possibility that pathogenic bacteria may also be present on the plates, which must be disposed of in a way which ensures destruction of the bacteria on them. See Attachment E for safe methods of disposal.

8 TESTING AT OFF-SITE LABORATORIES

Operators may use on-site laboratories or off-site laboratories. Where samples are sent off-site they should be stored no warmer than 5C (but not frozen) until transport, then kept chilled during transport the same day the sample is collected. The sample should arrive at the laboratory no warmer than 5C, though some tolerance to 10C is acceptable. If the sample arrives warmer than 10C it may still be analysed and recorded with reference made to the high temperature on arrival which may elevate counts, especially the TVC.

9 ADDITIONAL CUSTOMER REQUIREMENTS

Some operators will be required to conform with additional specific customer requirements for microbial testing or interpretation of results. In the present context "customer" may be an overseas regulatory authority or a private company (supermarket or importer).

10 INTERPRETATION OF RESULTS FOR CARCASES AND MEAT

Individual establishments should retain records of their results which are expressed as count/cm^2 of the surface area sponged for each category of livestock which is processed. If masses of meat are tested, a count/g is recorded. Plants should enter their test results onto a time graph. If data are entered into a system capable of providing trend analysis using a spreadsheet or purpose written software (eg. HACCP Monitor) this will allow microbiological data to be linked with other key indicators of process hygiene e.g. condition of livestock or meat hygiene assessments.

10.1 Guideline counts

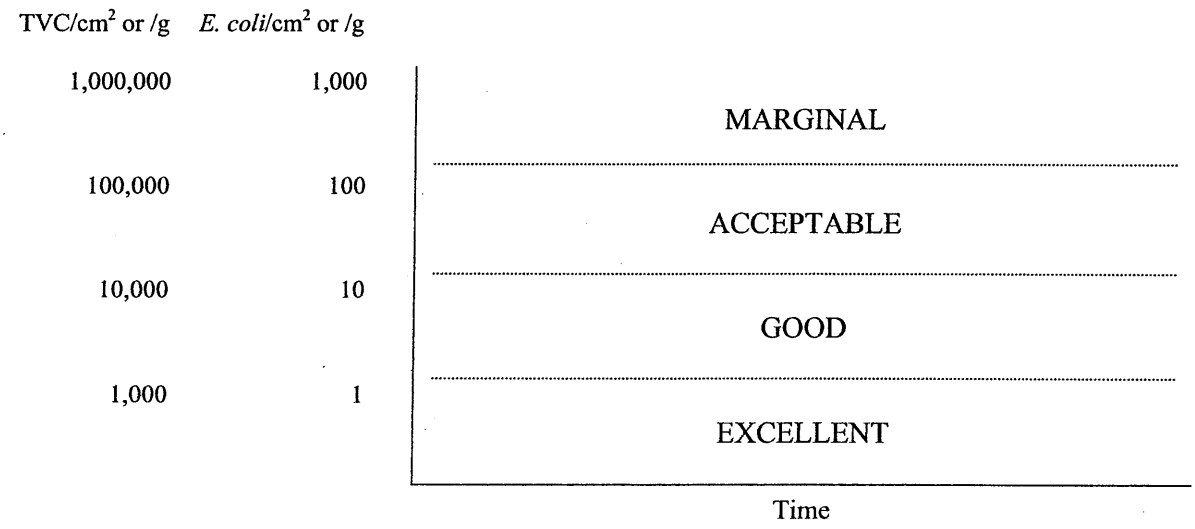
Product counts obtained using methods set out in these Guidelines signal whether microbiological conditions are within the normal range. Based on surveys of Australian meat (carcase and boxed meats), the following descriptions are used: Excellent, Good, Acceptable and Marginal for microbial levels listed below:

Category	TVC/cm^2 or /g	*E. coli*/cm^2 or /g
Excellent	<1,000	Not detected
Good	1,000-10,000	1-10
Acceptable	10,000-100,000	10-100
Marginal (Action required)	100,000-1,000,000	100-1,000

Experience shows that carcases and boneless meats from well-controlled processes will usually be in the Excellent or Good categories, with only occasional departures into the Acceptable category. It would be very unusual for these products to have TVC or E. coli count in the Marginal category and either count is a trigger for investigating reasons for high counts, and for Corrective Action.

10.2 Recording trends

QA staff should use test results to produce a time-course graph to show trends or high counts. Over a period of time each operation will generate its own microbiological baseline for each species. Generally, beef carcases will have lower counts than smallstock carcases.



If counts significantly above the company baseline occur, or if a general upward trend is seen, these should trigger an investigation of possible reasons, and Corrective Action. In the investigation, sampling needn't be confined to the sites stipulated in Attachments A-C. Sampling should be done to find where contamination is being brought onto the carcase surface, and sponging along cutting lines through the hide, into the body cavity and at the anus and weasand is especially useful. Remember to use a separate sponge for each site so that the count refers to a specific region or process.

The results of the investigation should be documented so that auditors or customers can be confident that the establishment is using microbiological monitoring in process improvement.

10.3 Reasons for high counts

Reasons for high counts include:

1. Dirty or dusty stock
2. Poor dressing practices
3. Poor equipment
4. Poor handling practices
5. Poor chiller management
6. Poor cleaning of facilities and equipment

11 Useful references for microbiological testing

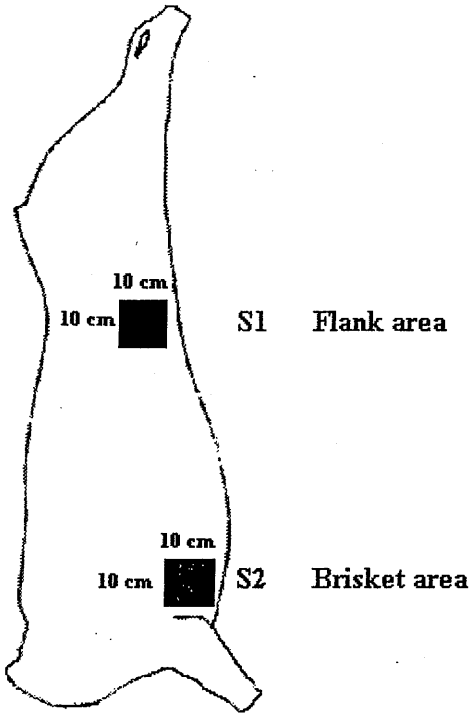
The following booklets contain useful information:

"Microbiological testing for the meat industry" available from MLA.

"Handbook for microbiological testing in food premises" (HB224:2001) available from Standards Australia.

Attachment A - Beef Carcase Sampling

Sampling Location for TVC and *E. coli* testing for Steer/Heifer and Cow/Bull carcases

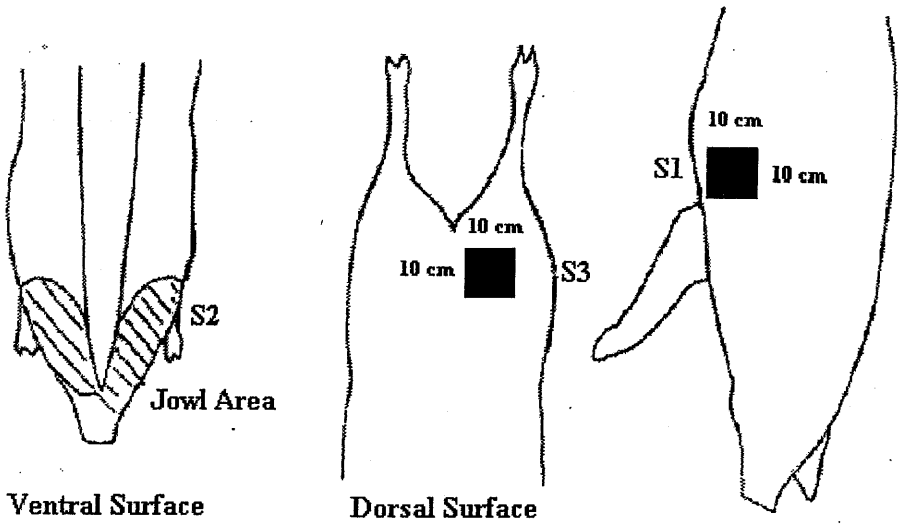


Swabbing sites 10 cm x 10 cm = 100 cm^2

Total area swabbed = 200 cm^2

Attachment B - Pig Carcase Sampling

Sampling location for TVC and *E. coli* testing for Pigs

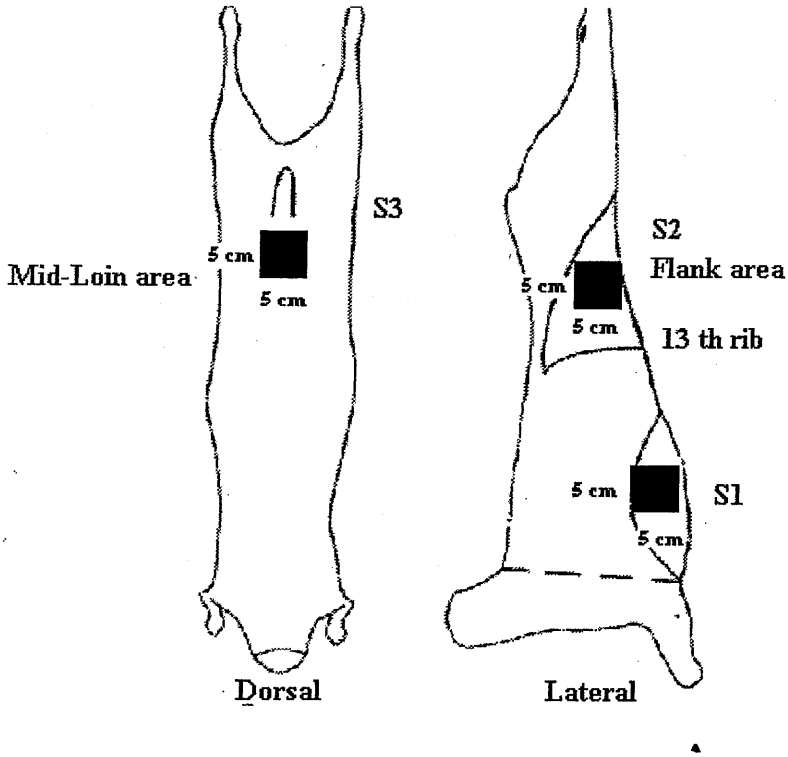


Swabbing site 10 cm x 10 cm = 100 cm^2

Total area swabbed = 200 cm^2 (head-off) or 300cm^2(head-on)

Attachment C - Sheep, Calves, Goats and Deer Carcase Sampling

Sampling location for TVC and *E. coli* testing for Sheep, Lambs, Calves, Goats, Deer.



Swabbing site 5 cm x 5 cm = 25 cm^2

Total area swabbed = 75 cm^2

Attachment D - How to collect samples from carcase surfaces

Materials

1. Sterile sponge in sterile bag or any other equivalent system approved by the Controlling Authority
2. Sterile diluent (Peptone water or Butterfields)
3. Template (where required by the Controlling Authority)
4. Sterile gloves
5. Sanitising solution (where steriliser facilities not available)
6. Container for carrying supplies

Collection

A sponge (which usually comes dehydrated in a sterile bag) is used to remove bacteria from sampling sites on the carcase without causing any damage to the product.

As much preparation as possible should be done in the laboratory.

Laboratory preparation

1. Label all sponge bags and accumulate all equipment.
2. Remove the cap from the diluent bottle, being careful not to touch the bottle opening.
3. Pour about half the diluent (10-15 mL) into the sponge bag.
4. Close the top of the bag by pressing the wire closure together.
5. Use hand pressure from outside of the bag and massage the sponge until it is hydrated.
6. With the bag still closed, carefully push the moistened sponge to the upper portion of the bag orienting one narrow end of the sponge up toward the opening of the bag. Don't open the bag or touch the sponge with your fingers.
7. While holding the bag, strip excess fluid from the sponge using hand pressure from the outside. The whole sponge should sit in the bag.

Sampling in the plant

1. Where a template is specified by the Controlling Authority, sanitise it and locate the sampling sites.
2. Open the bag containing the sponge, being careful not to touch the inner surface of the bag with your fingers. The wire closure at the top of the bag should keep the bag open.
3. Put on a pair of sterile gloves.
4. Remove the moistened sponge from the bag with the thumb and fingers (index and middle) of your sampling hand.
5. With your other hand, mark the sampling site with the template (where required).
6. Wipe the sponge over the sampling area (10 cmx10 cm) approximately 10 times in the vertical and 10 times in the horizontal directions. The pressure of sponging is important and should be as if you are removing dried blood from the carcase. However, the pressure should not be so hard as to crumble or destroy the sponge.
7. Sponge the other sampling site, using the same side or surface of the sponge used to swab previous site (in case of pigs or for small stock where three sites are to be sampled, use the "clean" surface or side {the side that was not previously used to swab other area of the sponge).

8. After swabbing all the sites, place the sponge back in the bag, taking care not to touch the sponge to the outside of the sample bag.

Completion in laboratory

1. Add the remaining diluent (10-15 mL) to the bag to bring the total volume to 25 mL.
2. Expel excess air from the bag containing the sponge and fold down the top edge of the bag 3 to 4 times to close. Secure the bag by folding the attached wire tie back against the bag.
3. If samples are to be analysed at an On-Site Laboratory, begin sample preparation.
4. If samples are to be analysed at an Outside (OFF-SITE) Laboratory, follow procedures in Attachment F.

Attachment E – How to carry out plating, count bacterial colonies and calculate the count/cm^2

1. Label plate or Petrifilm and record the following information in the sample book:
   - Date and time of sample
   - Species
   - Body number
   - Who sampled
2. Mix sample thoroughly by massaging sponge and diluent thoroughly for 15-20 seconds..
3. Pipette 1mL from the sponge bag onto the culture plate or Petrifilm.
4. Carry out the dilution technique by pipetting 1mL from the sponge bag into 9mL dilution blanks and carry out Step 3 for each dilution.
5. Incubate at 37°C for *E. coli* and at 20-25°C for 72 hours for TVC.
6. Examine *E. coli* colonies at 24 and 48 hours.
7. Count *E. coli* colonies after 48 hours and Total bacterial colonies after 72 hours. Optimum counting range is 30-300 on Petri dishes and 15-150 on Petrifilms.
8. Dispose of all sampling material by an effective method (see below).
9. Calculate the count/cm^2 and input data to the software package.

Calculating the count/cm^2

1 Counting direct from bag

Colony forming units (CFU)/cm^2 = Number of Colonies x $\frac{\text{Volume of diluent in bag}}{\text{Area sampled}}$

Example 1:

TVC count for beef carcase is 120 $\quad \text{cfu/cm}^2 = \frac{120 \times 25}{200} = 15/\text{cm}^2$

Example 2:

TVC count for sheep carcase is 120 $\quad \text{cfu/cm}^2 = \frac{120 \times 25}{75} = 40/\text{cm}^2$

2 Counting when dilution technique has been used

Colony forming units (CFU)/cm^2 = Count x $\frac{\text{Volume of diluent in bag x Dilution}}{\text{Area sampled}}$

Example 1:

TVC count for beef carcase is 50 and 2 dilution blanks were used (100x dilution)

$$\text{cfu/cm}^2 = \frac{50 \times 25 \times 100}{200} = 625/\text{cm}^2$$

Example 2:

TVC count for sheep carcase is 90 and 4 dilution blanks were used (10,000x dilution)

$$\text{cfu/cm}^2 = \frac{90 \times 25 \times 10{,}000}{75} = 300{,}000/\text{cm}^2$$

Disposal of Plates

Incubated plates showing colonies of bacteria must be disposed of safely, since the bacteria on the plate may be pathogenic.

Plates may be disposed of by:

1. Autoclaving
2. Incineration in an enclosed furnace
3. Treatment in a disinfectant bath
4. Collection from a Biohazard bin by a registered company

Attachment F - Transport of Samples

Where samples are to be transferred to an off-site laboratory for analysis the following procedures are to be used:

1. Samples are dispatched on the day of collection and analysed no later than the day following collection
2. Samples are maintained at refrigerator temperatures until shipped - samples must not be frozen
3. When samples arrive warmer than 10° +/- 1°C or late they may be analysed, but note should be made on the test report.
4. Bags containing sample sponges are secured and enclosed within a second firmly closed bag
5. Samples should be transported in a rigid plastic insulated container.

Packing procedure

1. Place a frozen gel pack in the bottom of the container
2. Place a corrugated cardboard divider above the gel pack
3. Place the sample(s) on the divider - crushed paper may be used to protect the sample(s) and hold them upright
4. Place a second divider above the samples
5. Place a second (and/or third) gel pack above the divider
6. Fill the vacant space with crushed paper
7. Seal the container securely with adhesive tape
8. Label as "meat samples"
9. Tick the "Does not contain dangerous goods" box on the consignment note

Dividers are used to prevent contact freezing of the samples. Sufficient gel packs must be used to ensure that the samples arrive at the laboratory ideally no warmer than 5C, with a tolerance up to 10C.

Each plant should validate their procedure by testing the temperature of a test sample after being held in a shipping container for 24 hours and 36 hours at ambient temperatures.