

**AUSTRALIAN STANDARD FOR CONSTRUCTION OF PREMISES  
AND HYGIENIC PRODUCTION OF POULTRY MEAT  
FOR HUMAN CONSUMPTION**

**APPENDIX A**

**MICROBIOLOGICAL TESTING FOR PROCESS MONITORING  
IN THE POULTRY MEAT INDUSTRY**

**GUIDELINES**

## 1. INTRODUCTION

The Australian Standard for Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption require that microbiological testing be carried out to verify the effectiveness of the process, stating that “Work and product services must be monitored on a regular basis to verify the HACCP program”.

The Standard requires:

1. Testing of product and working surfaces.
2. Keeping of records to enable benchmarks to be set.
3. Identifying trends over time.
4. Using microbiology as a tool to ensure that the process is delivering good results on a continuous basis.

The Standard specifically excludes using microbiology testing:

1. To judge wholesomeness of individual pieces of product.
2. To confirm freedom or absence of specific microorganisms.

Two important external influences must be considered in setting microbiological testing methods for the Australian industry:

1. The application of the Codex Alimentarius definition of critical control points (CCP's) has reduced the number of CCP's. As a result, microbiological testing embraces all aspects of the poultry meat processing chain, rather than monitoring merely the CCP's.
2. Overseas regulatory aspects have been introduced for certain markets.

The purpose of the present document is to recommend microbiological testing methods which can be used by poultry meat companies which service either or both domestic and export markets without significantly adding to their testing costs.

## 2. SCOPE

These Guidelines apply to:

Whole carcasses and pieces of poultry meat, the processing of which is required to comply with the Australian Standard for Construction of Premises and Hygienic Production of Poultry Meat for Human Consumption.

The Guidelines do not apply to further processed poultry meat products such as smallgoods and value-added products.

### 3. DEFINITIONS

Controlling Authority	An authority having responsibility for administering principal and subordinate legislation covering meat production at Commonwealth, State or Territory level
Corrective Action	Action taken by a poultry 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
Guideline Count	A microbiological criterion used to monitor a process to signal whether microbiological conditions are within the normal range
Process Review	A review of processes used in the production of poultry 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 poultry meat processor from product produced at the premises and from work surfaces at that premises
Standard Method	A sampling and/or testing method published by Standards Australia
Test Methods	Methods prescribed for taking microbiological samples from poultry meat and poultry meat products and testing these samples for the presence of specified microorganisms

### 4. RESPONSIBILITIES

#### 4.1 Controlling Authorities

Controlling Authorities must ensure that sampling and testing programs are undertaken to meet these Guidelines and will advise poultry meat processors of any changes to sampling plans or testing methods which eventuate from reviews of microbiological sampling data.

#### 4.2 Poultry Meat Processors

Poultry meat processors will:

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 available results to the Controlling Authority or its agents.

## **5. REASONS FOR DOING MICROBIOLOGICAL TESTING**

The reasons why a poultry meat processor is required to carry out microbial testing include:

1. Satisfying regulatory requirements.
2. Verifying the HACCP system.
3. Identifying areas of poor performance to enable corrective action to be taken.
4. Gauging the effectiveness of clean down.
5. Investigating contamination entry points along the process line.
6. Surveying the hygiene status of the different products manufactured.
7. Providing a customer with information on product quality.
8. Assessing product against a national or international benchmark.

Several methods of sampling and testing are available. However, to enable valid comparisons of results between establishments and over time, testing procedures must be standardised.

## **6. ASSESSING WORK SURFACES**

Testing of work surfaces is performed to check the effectiveness of clean down.

### **6.1 Sampling and Testing**

Samples for testing should be collected before commencement of operations with the aim of covering all important work surfaces on a fortnightly basis.

A selection of work surfaces will be tested at least weekly for TVC at 20-25°C, as an indicator of cleaning effectiveness.

Methods include swabbing and plating, or contact plating provided that the method chosen is applied consistently.

Testing should be rotated around the plant to provide an overall coverage over time.

Two basic test methods for estimating total viable counts on work surfaces exist:

1. Simple methods

Petrefilm, 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 surfaces will be picked up. After incubation for 2-3 days a count can be made of the number of bacteria/cm<sup>2</sup> on the medium reflecting the number on the initial work surface. The count will tell the operator whether the clean down was Satisfactory (6 colony forming units/ cm<sup>2</sup> or less) or Unsatisfactory (more than 6 colony forming units/ cm<sup>2</sup>). A swab technique is preferred for premises in which cutting up and boning out is the major activity.

## 2. Complex methods:

ATP bioluminescence methods are increasingly being used in the food industry, although their uptake in the meat industry has been slow. An ATP machine allows managers to monitor clean down in real time, which can give significant savings in cleaning costs.

The process involves the operator swabbing a defined area of work surface and placing the swab in the ATP instrument, which provides a numerical read-out. The number is related to the amount of organic matter, including animal debris and bacteria, remaining on the work surface. There should be none on a properly cleaned surface. The read out is interpreted according to the manufacturer's instruction on whether the surface has been satisfactorily cleaned.

## 3. Detailed methods:

Detailed methods for Contact Plate Technique and Swab Technique are provided in Attachment 1.

## 6.2 Corrective Action

Corrective action for poorly cleaned surfaces should be developed and those surfaces monitored in particular in future testing, measuring the effectiveness of the corrective action. Corrective action can include:

Review cleaning program:

- cleaning method
- chemicals (used as per manufacturer's specifications)
- program being followed
- effectiveness of hygiene monitoring reports
- intensive follow up monitoring (at least once per week) until two consecutive satisfactory results achieved
- consult chemical supplier.

## 7. ASSESSING POULTRY MEAT AND POULTRY MEAT PRODUCTS

Testing of products for Total Viable Count (TVC) will be carried out. Other target organisms may be specified by overseas regulators for shipment to their markets, or may be specified by Australian commercial customers (eg *Escherichia coli* biotype 1, *Salmonella* spp., *Campylobacter* spp.). In general, testing shall be carried out on:

1. Poultry meat surfaces – carcasses and pieces have microorganisms mainly on the surface, which must be removed for counting.
2. Masses – in the case of minced and emulsified products, the bacteria are distributed throughout the product and bacteria must be removed from the mass for counting.

In relation to poultry meat products, this section sets out requirements for products, which are the output of a process. Incoming raw materials used in the process may also need to be tested to verify the outcome of the HACCP plan applied to the process. Poultry meat processors will need to make the decision to test incoming materials in consideration of the requirements of their HACCP plan.

## 7.1 Sampling and Testing

Sampling and testing of product surfaces and whole poultry are to be carried out by methods approved by the Controlling Authority.

Details are provided in Attachment 2.

### Sampling Frequency

Plants will establish a satisfactory microbiological baseline level by sampling two whole birds per shift per processing day as follows:

	Week 1	Week 2	Week 3	Week 4
First Month	2 Birds per Shift	2 Birds per Shift	2 Birds per Shift	2 Birds per Shift
Second Month		2 Birds per Shift		2 Birds per Shift

When plants have demonstrated a satisfactory baseline level, the sampling frequency may be reduced to monthly. By contrast if microbiological levels are not consistently controlled, the sampling frequency will be intensified until the problem has been solved and a satisfactory baseline established.

Sampling may be clustered to one or more processing days of the week unless otherwise directed by the Controlling Authority as long as, over time, samples represent the overall throughput.

Very Small Premises (VSPs), defined as processing less than 10,000 birds per week, to sample five birds per shift with the collection of birds spaced out equally over the length of the shift. Sampling should take place every four months (this means 15 birds will be sampled per year). Where a significant change in the process takes place or the results of an audit indicate that processing is not delivering acceptable results the sampling regime can be adjusted to require a greater frequency of sampling as determined by the controlling authority.

## 7.2 Corrective Action

A product surface count exceeding  $1.5 \times 10^6$  microorganisms/cm<sup>2</sup>, using either the Rinse Technique or Skin Maceration Technique indicates unsatisfactory process control. Corrective action should focus on reviewing processes including examination of the hygiene status of birds, specific sites on the birds and chilling practices, for example, to determine the origin of the high counts. Corrective action must be documented and made available to controlling authority auditors.

## **8. MICROBIOLOGICAL SAFETY**

Culture plates must always be handled and stored to prevent any bacteria on the plate from coming in contact with people, since the bacteria on any plate may be dangerous. Plates must also be disposed of in a way, which ensures destruction of the bacteria on the plate.

Plates may be disposed of by:

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

## **9. TESTING AT OFF-SITE LABORATORIES**

Operators may utilise on-plant laboratories or off-site laboratories. Where samples are sent off-plant the samples must be maintained frozen below 0°C or at 4°C ± 1°C or colder until transport, then shipped refrigerated (4°C ± 1°C or colder) via an overnight courier the same calendar day the sample is collected. The sample must arrive at, and be analysed by, the laboratory the day after the sample is collected (refer to Attachment 3 for sample transport to external laboratories).

## **10. ADDITIONAL CUSTOMER REQUIREMENTS**

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

## **11. INTERPRETATION OF RESULTS FOR WHOLE BIRDS AND POULTRY PIECES**

Individual establishments should retain records of their results for at least 12 months. Results shall be expressed in terms of count/cm<sup>2</sup> of the surface area tested. Plants should enter their test results into a system capable of providing trend analysis using a spreadsheet or purpose written software (eg HACCP Monitor).

## 11.1 Guideline Counts

Australia has not set specific ranges but regulators will be looking to ensure that continuous improvement is achieved and that action is taken by processors whenever a high level is detected. During the last 23 years, there have been numerous surveys of the microbiological quality of Australian chicken meat carried out by the predecessor committees of the Chicken Meat Committee of the Rural Industries Research and Development Corporation as well as the Australian Poultry Industries Association. Based on these surveys, the following are the guidelines: Excellent, Good, Acceptable and Marginal with the actual microbiological levels for each category listed in the table:

Category	TVC/cm <sup>2</sup>
Excellent	<5,000
Good	5,000-50,000
Acceptable	50,000-500,000
Marginal	500,000-1,500,000

## 11.2 Recording Trends

Records shall be kept to enable benchmarks to be set and trends over time to be identified to indicate whether plant hygiene is satisfactory. For valid comparison of test results obtained at different times from a surface or product, it is essential that the same test technique be used each time.



## Attachment 1

### Sampling and Testing Methods for Work Surfaces

Sampling and testing of work surfaces shall be carried out by approved methods equivalent to those described below, which have been taken from the Australian Standard AS2998-1987, Cleaning and Sanitising of Plant and Equipment in the Poultry Processing Industry - Appendix B.

#### **Method A - Contact Plate Technique (AS2998 - 1987 Appendix B 8.4)**

Complete **each** of the following steps:

1. Prepare contact plates by filling the plate with sterile molten agar medium to form a slightly convex surface. Replace the cover and allow the agar to solidify. Alternatively, the use of off-the-shelf products such as Petrifilm is suitable for this technique (other equivalent products are also available).

Note: Where a final rinse has not been used to remove all traces of sanitizer from the surface (or where it may have been below optimal) a suitable neutralizer should be incorporated in the surface contact agar e.g. 0.5 percent polysorbate 80 is used to neutralize substituted phenolic sanitizers, and 0.07 percent soy lecithin is used to neutralize quaternary ammonium compounds. (*Note: Polysorbate 80 is available commercially as "tween 80"*).

2. To sample the surface, remove the contact plate cover and bring the agar into contact with the surface with a slight rolling action. Replace the cover.

Note: The agar surface should not be touched by the operator.

3. Using another sterile contact plate repeat Step 2 to take a replicate sample at a site adjacent to the first site.
4. Incubate the samples at 25°C for up to 72 hours.
5. Examine the plates and when the colonies are large enough to count, record the number of colonies present on the replicate sample contact plates as the number of colonies per specified area. e.g. square centimetre.

Note: A surface count of 6 microorganisms/cm<sup>2</sup> using this method indicates satisfactory cleaning. If the surface count exceeds 6 microorganisms/cm<sup>2</sup> then the corrective action for work surfaces should be followed (see Section 6.2).

## **Method B - Swab Technique (AS2998 - 187 Appendix B 9)**

This is the preferred method for premises in which cutting up and boning out is the major activity. This technique can be performed using cotton wool swabs and plating out or, alternatively, by using a swab kit.

**Using cotton wool swabs and plating out**, complete **each** of the following steps.

1. Sterilise the template by dipping it in methylated spirits and flaming.
2. If the surface to be tested is dry, moisten the sterile cotton swab with sterile peptone water or 0.85% saline.
3. Swab the defined area of test surface by rubbing firmly using parallel strokes with slow rotation of the swab. Repeat using parallel strokes at right angles to the first set.
4. Streak the swab over the surface of a sterile nutrient agar plate in a N-S and E-W direction rotating the swab during plating.
5. Repeat steps 3 and 4 to sample several adjacent areas using a separate swab for each.
6. Incubate the plates at 25°C for up to 72 hours.
7. Examine the plates and when the colonies are large enough to count, record the number of colonies present on the replicate sample contact plates as the number of colonies per specified area. e.g. square centimetre.

Note: A surface count of 6 microorganisms/cm<sup>2</sup> using this method indicates satisfactory cleaning. If the surface count exceeds 6 microorganisms/cm<sup>2</sup> then the corrective action for work surfaces should be followed (see Section 6.2).

**Using a swab kit**, complete **each** of the following steps:

1. Withdraw swab from the buffer solution and roll the tip on the inside of the container to wring out the excess buffer.
2. Swab the defined area of the test surface by rubbing firmly using parallel strokes with slow rotation of the swab. Repeat using parallel strokes at right angles to the first set.
3. Insert the swab in the buffer container and shake. Discard the swab.
4. Insert the sampler in the buffer and leave it there for 30 seconds.
5. Remove sampler and shake vigorously to remove excess buffer.
6. Return the sampler to its original container and incubate at 25°C for up to 72 hours.
7. Examine the plates and when the colonies are large enough to count, record the number of colonies present on the replicate sample contact plates as the number of colonies per specified area. e.g. square centimetre.

Note: A surface count of 6 microorganisms/cm<sup>2</sup> using this method indicates satisfactory cleaning. If the surface count exceeds 6 microorganisms/cm<sup>2</sup> then the corrective action for work surfaces should be followed (see Section 6.2).

## Attachment 2

### Sampling and Testing Methods for Product

Sampling and testing of product surfaces and whole poultry shall be carried out by approved methods equivalent to those described below, which have been taken from the Australian Standard AS1766.3.2 -1979, Methods for the Microbiological Examination of Food, Part 3 Section 2, Poultry.

#### Method A - Rinse Technique (AS 1766.3.2.6.2)

This procedure may be used for chilled or frozen poultry, whole or portions, weighing up to 2kg, by completing **each** of the following steps.

1. If frozen poultry is being tested thaw any frozen poultry samples at 2°C to 5°C for a period not exceeding 24 hours.
2. Record the mass of whole birds or the number and collective mass of portions of each type, the date, the name of the person responsible for sampling and the time interval between time of original sampling and commencement of testing.
3. Transfer the poultry meat and any accompanying fluid from its original packaging material into a plastic bag of suitable size.
4. Add 500 ml of 0.1 percent peptone solution.
5. Remove approximately half the air from the bag by massaging around the sample from the closed end to the open end. Tie the open end.
6. Shake and massage the sample vigorously for 2 minutes ensuring thorough rinsing of the abdominal cavity.
7. Release the rinse fluid into a sample container by aseptically cutting off a corner of the bag with sterilised scissors and allowing the fluid to run into the container. The rinse fluid is the first dilution.
8. Pipette 1 ml of the rinse fluid onto an agar plate or Petrifilm.
9. Carry out a series of dilutions by pipetting 1 ml from the rinse fluid into 9 ml peptone solution dilution blanks to establish an optimum counting range of 30-300 colonies on agar and 15-150 on Petrifilm.
10. Pipette 1 ml of each dilution onto an agar plate or Petrifilm.
11. Incubate at 30°C or 20-25°C ± 1°C for 72 hours.
12. Count the number of colonies.
13. Calculate the surface area of the poultry meat in square centimeters using the following formulas:

(a) Whole Birds		(b) Portions	
Chickens	$0.87m + 635$	Wing	$86n + 1.41m$
Turkeys		Back	$94n + 1.11m$
(i) less than 7 kg	$0.45m + 1293$	Breast	$137n + 0.84m$
(ii) over 7 kg	$0.13m + 3480$	Thigh and drumstick	$60n + 0.90m$
Ducks	$0.81m + 696$		

Where

$m$  = total mass of  $n$  pieces, in grams

$n$  = number of pieces

14. Calculate the microorganisms per square centimeter of surface area from the rinse fluid using the following formula:

$$\text{Colony forming units (CFU)/cm}^2 = \frac{\text{Number of colonies} \times \text{volume of rinse fluid (500ml)}}{\text{Surface area of poultry meat}}$$

Calculate the microorganisms per square centimeter of surface area, when the dilution technique has been used, by the following formula:

$$\text{Colony forming units (CFU)/cm}^2 = \frac{\text{Number of colonies} \times \text{volume of rinse fluid (500ml)} \times \text{dilution}}{\text{Surface area of poultry meat}}$$

### **Method B - Skin Maceration Technique (AS 1766.3.2.6.3)**

This procedure may be used for uncoated poultry, whole or portions, upon which a sufficient and suitable portion of skin has been retained, by completing each of the following steps.

1. Place a 10 cm<sup>2</sup> metal template against the breast skin of whole poultry or against the appropriate section of a poultry portion and excise a skin tissue area of 10 cm<sup>2</sup>.
2. Remove the skin tissue portion with forceps and place separately in tarred blender containers.
3. Determine and record the mass of skin tissue, the date, the name of the person responsible for sampling and the time interval between time of original sampling and commencement of testing.
4. Blend the portion of skin tissue in 100 ml of 0.1 percent peptone solution at high speed for 60 seconds or until the solid particulate matter is reduced to a fine state. This is the first dilution.
5. Pipette 1 ml of the rinse fluid onto an agar plate or Petrifilm.
6. Carry out a series of dilutions by pipetting 1 ml from the rinse fluid into 9 ml peptone solution dilution blanks to establish an optimum counting range of 30-300 colonies on agar and 15-150 on Petrifilm.
7. Pipette 1 ml of each dilution onto an agar plate or Petrifilm.
8. Incubate at 30°C or 20-25°C ± 1°C for 72 hours.
9. Count the number of colonies.
10. Calculate the microorganisms per square centimetre of surface area from the first dilution using the following formula:

$$\text{Colony forming units (CFU)/cm}^2 = \frac{\text{Number of colonies} \times \text{volume of rinse fluid (100ml)} \times \text{dilution}}{\text{Surface area of skin tissue (10 cm}^2\text{)}}$$

Calculate the microorganisms per square centimetre of surface area, when the dilution technique has been used, by the following formula:

$$\text{Colony forming units (CFU)/cm}^2 = \frac{\text{Number of colonies} \times \text{volume of rinse fluid (100ml)} \times \text{dilution}}{\text{Surface area of poultry meat}}$$

## Attachment 3

### 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 not later than the day following collection.
2. Samples are maintained frozen or at refrigerator temperatures until shipped.
3. The laboratory is instructed to discard samples, which arrive warmer than  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$  or late (cannot be analysed the day following collection).
4. Bags containing samples are secured and enclosed within a second firmly closed bag.
5. Samples may be transported in a rigid plastic insulated container.
6. Frozen samples must remain at or below  $0^{\circ}\text{C}$  until ready for analysis.

### Packing procedure {tc "**Packing procedure**"}

#### *a. Chilled samples:*

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 the container as “poultry 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 at a temperature of  $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$  or colder.

Each plant should validate its 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.

#### *b. Frozen samples:*

Should be appropriately packed and transported in containers that can maintain the product temperature at  $0^{\circ}\text{C}$  or lower.