

# STANDARD OPERATING PROCEDURE FOR MICROBIOLOGICAL EXAMINATION OF POULTRY.

## 1. PURPOSE

To ensure that the microbiological examination of poultry, carried out as part of a plant “own check” programme for the control the general hygiene of food processing operations is carried out in accordance with Commission Regulation No. 2073/2005 on the microbiological criteria of foodstuffs.

## 2. SCOPE

This standard operating procedure (SOP) is applicable to the bacteriological examination of poultry for *Salmonella* spp, ACC and *Enterobacteriaceae* in slaughterhouses using the neck skin excision method.

Plant management are responsible for ensuring that personnel with designated responsibility for carrying out this procedure have received formal training.

Plant management are obliged to provide the necessary resources to facilitate and ensure the health and safety of personnel carrying out this procedure.

## 3. DEFINITIONS

**Mean Log = the arithmetic average of the logarithm (base 10) of individual carcass test results (in colony forming units per gram: cfu/g) for all carcasses sampled on a given day, see worked examples 4.3.4, 4.3.5 and 4.3.6.**

*Note: When computing the mean log value where the average bacterial counts may be less than 1, the logarithm of counts of less than 1 should be designated as 0 for the purpose of constructing the process control charts.*

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### 4. PROCEDURE

#### 4.1 Sampling method

4.1.1 A minimum of 15 carcasses should be sampled on each sampling session and a piece of approximately 10g of neck skin (Diagram A) removed aseptically from each carcass. The neck skin from 3 carcasses should be pooled before examination so that in total five composite samples are obtained per session. In total 50 sample results ( $n = 50$ ) derived 10 consecutive sampling sessions should be examined.

Diagram A: Aseptic removal of poultry neck skin sample.



*Note 1: An alternative sample site to the above may be used in consultation with the official veterinarian where it can be demonstrated that because of the production processes involved, other sites are likely to carry higher levels of contamination.*

*Note 2: It is imperative that the sample is taken under aseptic conditions and that the equipment used is sterile.*

*Note 3: The composite samples obtained may also be used as a source of sample material for pathogen testing (for example Salmonella), subject to the approval of the official veterinarian in charge.*

*Sterility can be achieved by:*

- 1) *Flaming the cutting equipment using 70% ethanol and a portable gas blow torch or cigarette lighter.*

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- 2) *Using disposable alcohol wipes.*
- 3) *Placing samples in sterile, peristaltic type, homogeniser bags or sterile sample containers.*  
*Other methods, which will achieve the same results, may be used.*

4.1.2 Sampling should be carried out after carcass chilling.

4.1.3 The carcasses are sampled on one day of each working week and to ensure that they are representative of the factory throughput, the day on which sampling is carried out should be altered each week.

#### **4.2 Sample maintenance, storage and transport**

4.2.1 Using aseptic techniques, a composite sample from each carcass is placed in a sterile container or bag prior to transfer to the laboratory.

4.2.2 Each sample container should be identified with the flock number or batch identification, time and date of sampling and the identity of the person who took the sample.

4.2.3 The sample container is stored between 0-4°C (e.g. using a coolbox) and then transported to the laboratory under chilled conditions (0-4°C).

N.B, The time between sampling and laboratory examination should not exceed 24 hours.

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**4.3. Laboratory examination:**

4.3.1 The analyses for aerobic colony count (ACC), *Enterobacteriaceae* and *Salmonella* spp. are carried out using the following ISO procedures:

- (a) ISO 4833:2003, Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 °C.
- (b) ISO 21528-2:2004 'Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – part 2: Colony-count method'
- (c) ISO 6579 'Horizontal method for detection of *Salmonella* spp.'

*Note 1: Methods other than the ISO methods described above may be used where they have been validated against the reference method or validated according to internationally accepted protocols and authorised by the Competent Authority. E. coli counts may be used instead of Enterobacteriaceae counts following approval by the Department of Agriculture and Food, and establishment of appropriate criteria. If methods other than the standard ISO methods given above are used, details must be provided for the incubation temperature and times, types of agar and inoculation volumes and reference to method used.*

*Note 2: Testing for Salmonella is a compulsory requirement in Commission Regulation No 2073/2005. Testing for Enterobacteriaceae counts and aerobic colony counts (ACC) is a voluntary requirement only but seen as indicator of good process hygiene.*

4.3.2 Laboratory results are recorded as colony forming units per gram (cfu/g) for each composite (three carcass) sample using the following formula:

$$\text{Cfu/g} = \text{Average cfu/plate} \times \text{dilution factor}$$

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Example: Average cfu/plate = 110 on  $10^{-1}$  dilution

$$\begin{aligned} \text{cfu/g} &= 110 \times 10 \\ &= 1100 \\ &= \mathbf{1100 \text{ cfu/g}} \end{aligned}$$

- 4.3.3 The logarithm of each result is obtained by taking the logarithm to base 10 ( $\log_{10}$ ) of cfu/g for each composite sample result.
- 4.3.4 The mean log is calculated by taking the logarithm to base 10 ( $\log_{10}$ ) of each individual composite test result and then calculating the arithmetic mean of these log values. An example of a calculation using data from five composite samples is as follows:

Composite no	Colony count (cfu/g)	logarithm to base 10 ( $\log_{10}$ )
1	20000	4.3
2	1485	3.2
3	300	2.5
4	450000	5.7
5	2500	3.4
Mean Log (arithmetic mean of log values)		3.8

- 4.3.5 The laboratory records should show the name and address of the laboratory carrying out the examination, the date of the examination, reference to the method

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used, the signature of the laboratory supervisor, identification of the sample (including date and hour of sampling) and the results. These should be reported as the cfu per plate used to calculate the number of cfu/g computed as in 4.3.4, 4.3.5 and 4.3.6 above. The *Salmonella* spp. results should be reported as presence/absence.

- 4.3.6 Laboratory records must be maintained for a period of not less than 18 months after the date of sampling and must be available on request by the veterinary officer in charge.

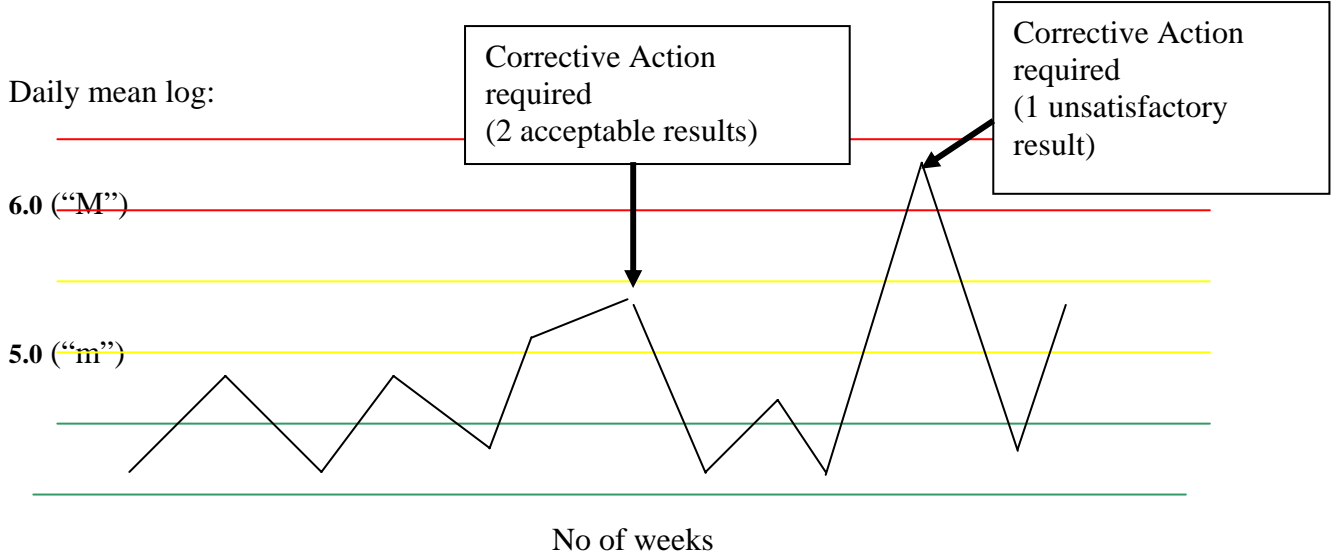
**4.4 Presentation/evaluation of results and process control**

- 4.4.1 Daily mean log values for ACC, *Enterobacteriaceae* and presence/absence of *Salmonella* spp. are presented in the form of the following Process Control Charts A, B and C. These provide ready identification where there is the need to take corrective action on the process.

Charts A and B presents data for daily mean log values and shows the acceptable (m) and the unsatisfactory (M) limiting criteria for the microbiological counts. Chart C presents data for 10 consecutive weeks where c is the maximum number of positive *Salmonella* samples allowed from total of 50 samples (5 pooled samples per week)

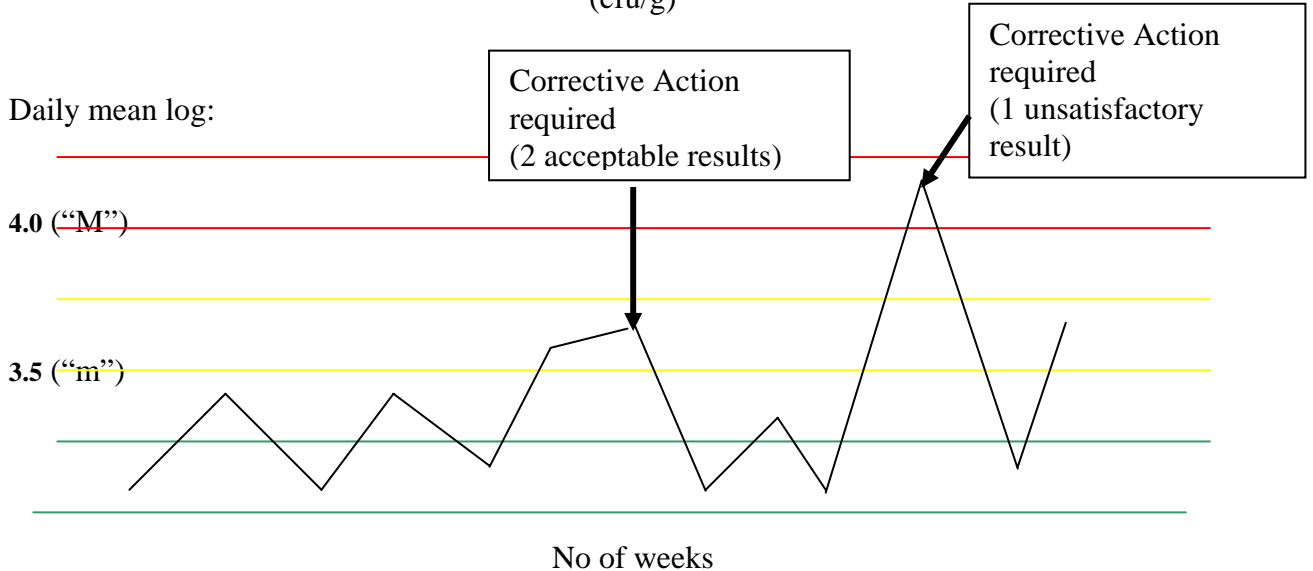
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Process Control Chart A: Aerobic colony count - daily mean log values of colony forming units (cfu/g):



Comments:  
Action taken:

Process Control Chart B: *Enterobacteriaceae* - daily mean log values of colony forming units (cfu/g)

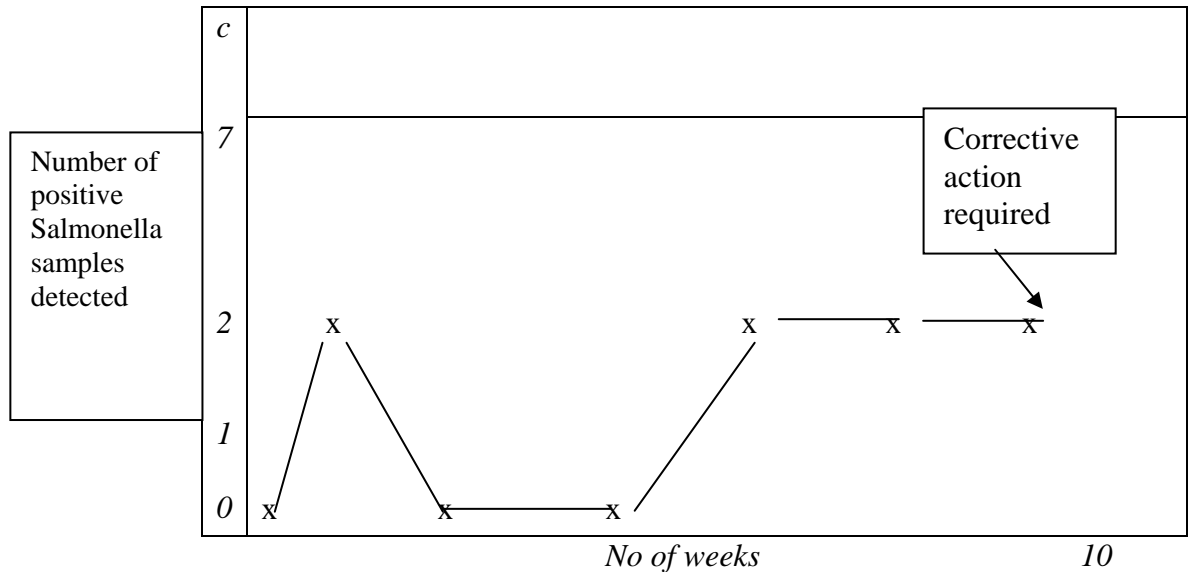


Comments:  
Action taken:

*Note: above plots are for illustrative purposes only*

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Process Control Chart C: Salmonella



4.4.2 Corrective actions:

Where one mean log result **on either chart** exceeds the unsatisfactory Range, or two consecutive results exceed the satisfactory Range, immediate corrective action is required to address the root cause of the problem (see Process Control Chart above). Where there is greater than 7 positive Salmonella results from 10 consecutive sampling sessions (50 samples in total) then correction action should be taken to address the problem. This action is documented after consultation with the responsible staff by the appropriate plant personnel.

*Note 1: Corrective actions should include: Review of flock hygiene, animal welfare and controls at producer level, review of transportation procedures, evaluation of operation cleanliness, improving working procedures/instructions, retraining, review of cleaning/effectiveness of disinfection materials and maintenance/cleaning equipment, improved supervision and review of controls at relevant critical control points, review of biosecurity measures on farms.*

*Note 2: In the case of Salmonella, the frequency of sampling may be reduced to fortnightly if satisfactory results are obtained for 30 consecutive weeks, subject to the agreement of the veterinary officer in charge.*

*Note 3: Sampling frequency may be increased where the results of sampling are unsatisfactory*



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Issued by: Appropriate plant personnel

*Note 4: when justified on the basis of risk analysis and consequently authorised by the competent authority, small slaughterhouses and establishments producing minced meat and meat preparations in small quantities may be exempted from these sampling frequencies. A sampling frequency of one day in every five days of slaughtering may be used for such premises.*

***The specific microbial limiting criteria for poultry to be used in the Process Control Chart (Criteria are based on historical baseline data submitted by poultry processing companies and research carried out by the Faculty of Veterinary Medicine, University College Dublin.)***

<i>Daily mean log values (cfu/g) for the neck sampling method</i>	<i>Satisfactory range</i>	<i>Acceptable range (m)</i>	<i>Unsatisfactory range (M)</i>
<i>ACC</i>	<i>≤ 5.0 log (100,000 cfu/g)</i>	<i>&gt;5.0 – 6.0 log (100,000- 1,000,000 cfu/g)</i>	<i>&gt; 6.0 log (1,000,000 cfu/g)</i>
<i>Enterobacteriaceae</i>	<i>≤ 3.5 log (3162 cfu/g)</i>	<i>&gt;3.5 – 4.0 log (3162 – 10,000 cfu/g)</i>	<i>&gt; 4.0 log (10,000 cfu/g)</i>

**Salmonella sampling plan:**

Food category	Sampling plan		Limits
	n	c	
Carcasses of broilers and turkeys	50	7	Absence in 25g of pooled neck sample

Where:

n – number of carcasses tested

c – number of carcasses above which corrective action as outlined in section 4.4.2 above must be applied.

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**5 REFERENCES**

ISO 4833:2003, Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 °C.

ISO 21528-2:2004 'Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – part 2: Colony-count method'

Commission Regulation (EC) No 2073/2005, on Microbiological Criteria for foodstuffs, 15<sup>th</sup> November 2005.

ISO 6579 'Horizontal method for detection of *Salmonella Sp.*'

Whyte, P., Bolton, D., O'Mahony, M., Collins, D., (2002). Development and application of Hazard Analysis Critical Control Point (HACCP) systems in commercial broiler production and slaughter, ISBN 1 90227 7627.