

STANDARD OPERATING PROCEDURE FOR MICROBIOLOGICAL EXAMINATION OF CARCASSES BY EXCISION – DESTRUCTIVE METHOD

Ref: Issue: 01

Issued by: Appropriate plant
personnel

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1. PURPOSE

To ensure that the microbiological examination of carcasses, 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 the requirements of 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 cattle, pigs and sheep carcasses for *Enterobacteriaceae* and Aerobic Colony Count (ACC) in slaughterhouses using the excision (destructive) 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, including for example, a platform device to enable rump samples to be taken from bovines.

3. DEFINITIONS

Since the logarithms of the microbial counts on carcasses are likely to be normally distributed, the Mean Log value is used and defined to be:

Mean Log = the daily mean of the log values taken by calculating the log (\log_{10}) of each individual test result and then calculating the arithmetical mean of these log values, see worked examples 4.3.4, 4.3.5 and 4.3.6.

Note: When computing the mean log value for Enterobacteriaceae 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 Four separate tissue samples, each of area 5cm² and totalling 20cm², are taken from each carcass at the following designated sites, also indicated in diagrams A, B, C.

The recommended sampling sites are:

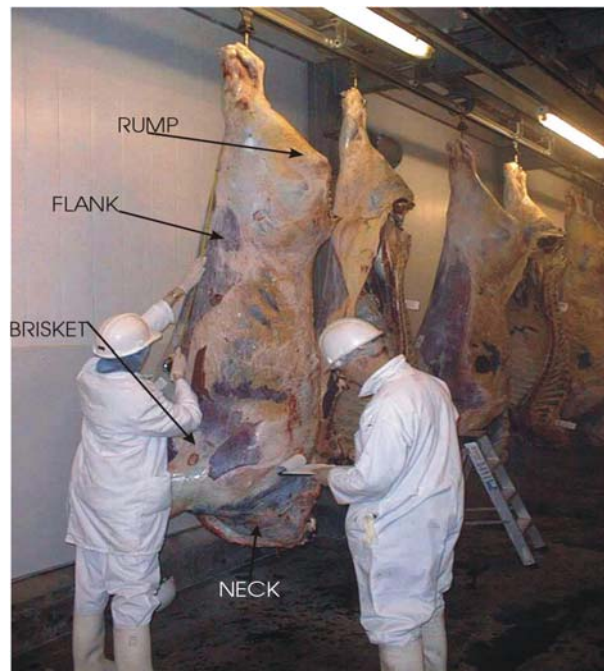
Cattle: neck, brisket, flank and rump

Pigs: back, jowl (or cheek), medial aspect of the ham, belly

Sheep: flank, lateral thorax, brisket and breast

Note: Alternative sample sites to the above may be used in consultation with the competent authority where it can be demonstrated that because of the slaughter technology involved, other sites are likely to carry higher levels of contamination.

Diagram A: Recommended sampling sites for cattle



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Diagram B: Recommended sampling sites for pigs

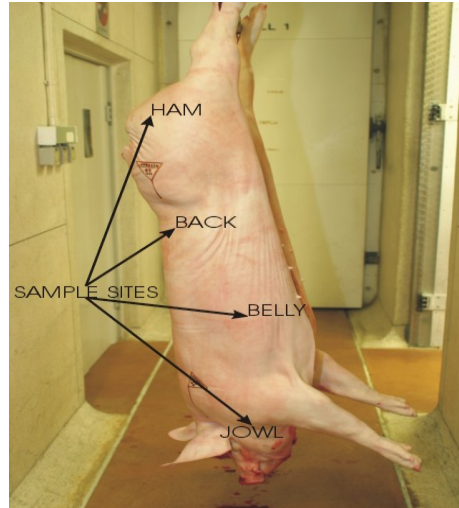
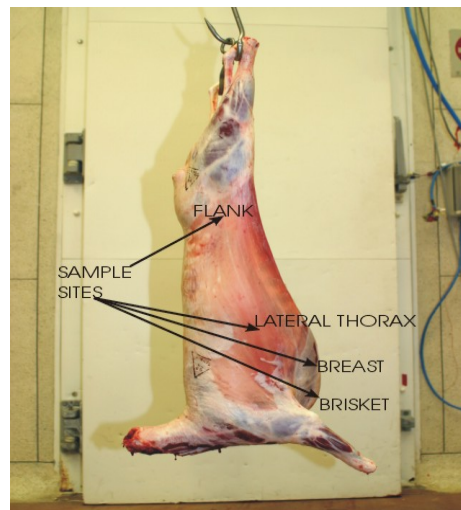


Diagram C: Recommended sampling sites for sheep



- 4.1.2. The samples are taken using a sterile cork borer (2.5 cm diameter) (Diagram D) or by cutting a slice of 5cm² and removal using a sterile scalpel and forceps (Diagram F). In either case the sample should have a maximum thickness of 2 mm.

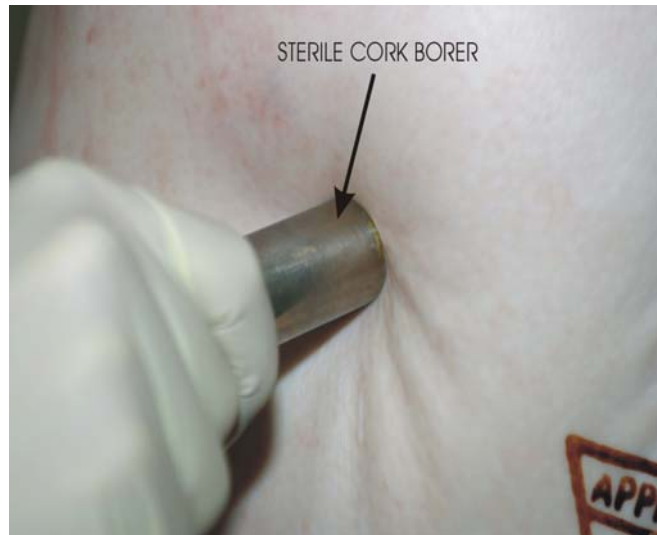
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Diagram D: Removal of sample from carcass surface using a cork borer.



Note: it is imperative that the sample is taken under aseptic conditions and that the equipment used is sterile (Diagrams D, E, F, G). Sterility can be achieved by:

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

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Diagram E: Sterilising a cork borer immediately before using it to sample carcasses

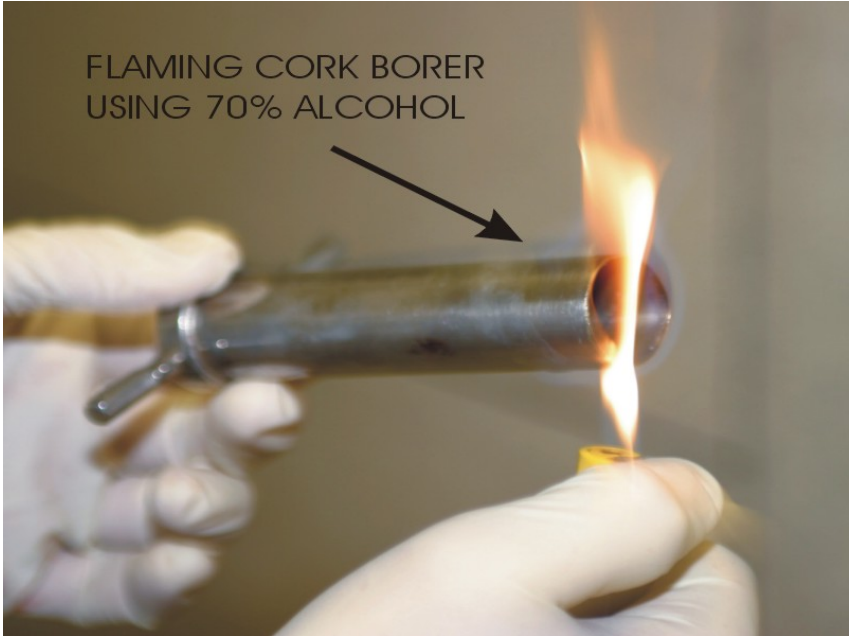
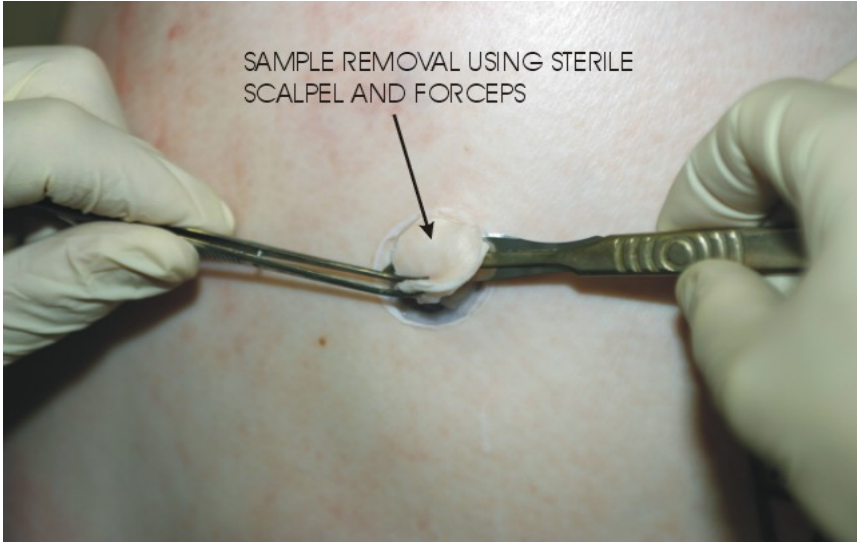


Diagram F: Excision of sample from a carcass surface



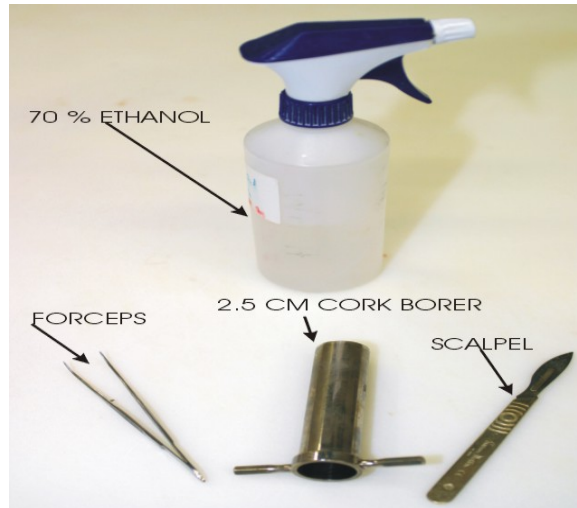
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Diagram G: Tools required (excluding lighter) for microbiological sampling of carcasses.



- 4.1.3 Sampling should be carried out after the completion of carcass dressing, but before the commencement of chilling.
- 4.1.4 5 carcasses are sampled on one day of each working week.
- 4.1.5 Samples are taken half way through the slaughter day, and to ensure that they are representative of the factory throughput, the day on which sampling is carried out should be altered each week.
- 4.1.6 The four individual samples from each sample site are pooled e.g. for cattle: neck, brisket, flank and rump samples are pooled.

4.2 Sample maintenance, storage and transport

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- 4.2.1 Using aseptic techniques, the pooled 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 carcass number, 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.

4.3. Laboratory examination:

- 4.3.1 Samples are homogenised using a peristaltic stomacher at a speed of 250 cycles/min for 2 minutes using 100ml of maximum recovery dilution medium (0.1% peptone, 0.85% sodium chloride solution). The suspension contained in the stomacher bag is not a dilution and is represented in the calculation as the 10^0 dilution.
- 4.3.2 Before plating, dilution should be carried out in 10 fold steps using the maximum recovery dilution medium.

Note: In preparing the dilutions 1ml is transferred from the 10^0 suspension into 9ml of diluent to give a dilution of 10^{-1} . This procedure is repeated for further dilutions using a fresh sterile pipette for each decimal dilution. The purpose of the dilutions is to ensure that the colony count per plate is in the range of 15-300 cfu.

- 4.3.3 The analyses for aerobic colony counts (ACC), and Enterobacteriaceae 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'

Note: Methods other than the ISO methods described above may be used where they have been validated against the reference method. Proprietary methods can be used if certified by a third party in accordance with the protocol set out in ISO

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standard 16140 or other internationally accepted protocols. If the food business operator wishes to use methods other than those described, they must be validated according to internationally accepted protocols and their use authorised by the competent authority. 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.

- 4.3.4 Laboratory results are recorded as colony forming units (cfu/cm²) for each pooled carcass sample (container) using the following formula:

$$\text{Cfu/cm}^2 = \frac{\text{Average cfu/plate} \times a \text{ (volume of original suspension)}}{b \text{ (total surface area e.g. } 5\text{cm}^2 \times 4 \text{ excisions)} \times \text{(dilution factor)}}$$

Example: Average cfu/plate = 85 on 10⁻¹ dilution (Excision sample)

$$\begin{aligned}\text{cfu/cm}^2 &= \frac{85 \times 100}{20 \times 10^{-1}} \\ &= \frac{85 \times 100 \times 10}{20} \\ &= \underline{\underline{4250 \text{ cfu/cm}^2}}\end{aligned}$$

4.3.5 The logarithm of each result is obtained by taking the logarithm to base 10 (log₁₀) of cfu/cm² for each pooled sample from each carcass sampled on the day.

- 4.3.6 The daily mean of the log values is taken by calculating the log (log₁₀) of each individual test result and then calculating the arithmetical mean of these log values. An example of a calculation using data from five carcasses is as follows:

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Carcass number	Colony count (cfu/cm ²)	(log ₁₀)
1	200000	5.3
2	1485	3.2
3	3000	3.5
4	4500	3.7
5	25000	4.4
Arithmetic average		4.0
Mean log		4.0

Note: in this example, the ACC falls within the acceptable range for cattle and sheep.

4.3.7 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 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/cm² of carcass surface area computed as in 4.3.4, 4.3.5 and 4.3.6 above.

4.3.8 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 of **both** ACC and *Enterobacteriaceae* are presented in the form of the following Process Control Charts A and B (presented on a single sheet). These provide ready identification where there is the need to take corrective action on the process.

Each chart presents data for mean log results and shows the acceptable (m) and the unsatisfactory (M) limiting criteria for the microbiological counts.

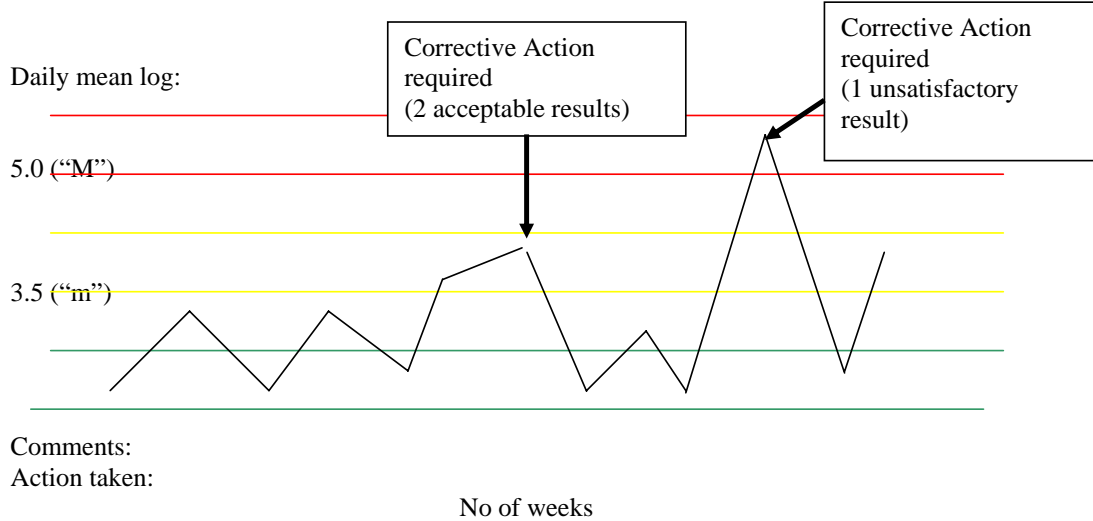
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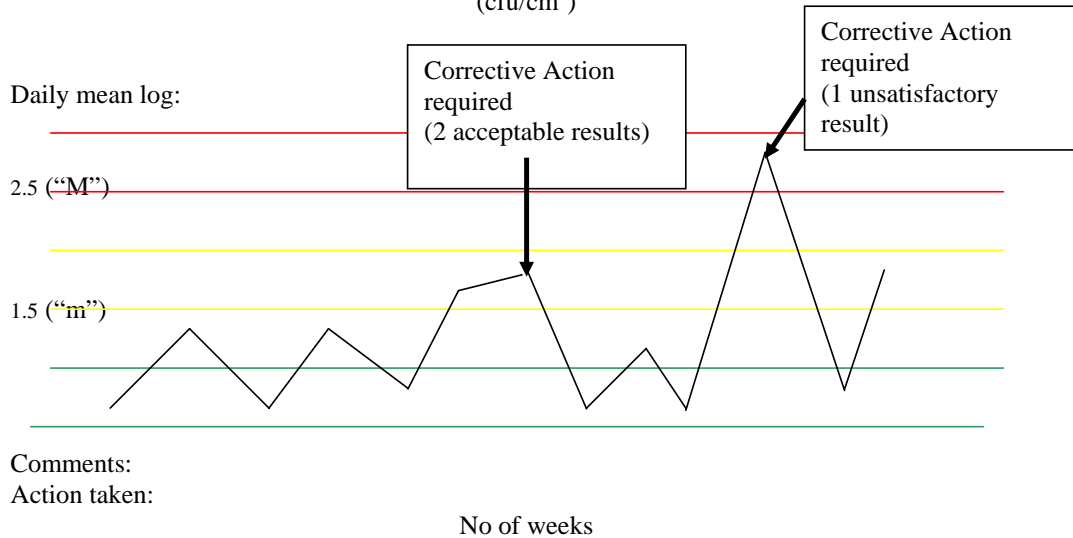
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Process Control Chart A: Aerobic colony counts- daily mean log values of colony forming units (cfu/cm²):



Process Control Chart B: *Enterobacteriaceae*- daily mean log values of colony forming units (cfu/cm²)



Note: above plots are for illustrative purposes only and the example given is for that for carcasses of cattle, sheep.

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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). This action is documented after consultation with the appropriate plant personnel.

Note: Corrective actions should include: evaluation of animal cleanliness, improving working procedures/instructions, retraining, review of cleaning/disinfection materials and maintenance/cleaning equipment, improved supervision.

4.4.3 Where test results are unsatisfactory and where corrective action does not eliminate the problem, pooling of samples is suspended until the problem is resolved.

Note 1: The frequency of sampling may be reduced to once per two week period, subject to the agreement of the veterinary officer in charge, where the average results are considered satisfactory following six consecutive weeks of sampling.

Note 2: Sampling frequency may be increased where the results of sampling are unsatisfactory

Note 3: 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.

The specific microbial limiting criteria to be used in the Process Control Chart are taken directly from the micro criteria regulation

Daily mean log values (cfu/cm ²) for the destructive method	Satisfactory range		Acceptable range (m)		Unsatisfactory range (M)	
	Cattle/Sheep/Goat/Horse	Pigs	Cattle/Sheep/Goat/Horse	Pigs	Cattle/Sheep/Goat/Horse	Pigs
ACC	≤3.5	≤ 4.0	> 3.5 - 5.0	> 4.0 -5.0	> 5.0	> 5.0
Enterobacteriaceae	≤ 1.5	≤ 2.0	> 1.5 – 2.5	> 2.0 -3.0	> 2.5	> 3.0

≤: less than or equal to, <: Less than, >: greater than.

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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 of Foods, 15th November 2005.