STANDARD OPERATING PROCEDURE FOR MICROBIOLOGICAL EXAMINATION OF CARCASSES BY WET/DRY SWABBING

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 .

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2. SCOPE/RESPONSIBILITY:

This Standard Operating Procedure (SOP) is applicable to the bacteriological examination of cattle, sheep and pig carcasses in slaughterhouses using wet/dry swabbing method.

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

This SOP applies to the microbiological examination of carcasses for aerobic colony counts (ACC) and Enterobacteriaceae only.

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 actual microbial counts on carcasses are likely to be normally distributed, the Mean log value is 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.

4. PROCEDURE

4.1 Sampling method

4.1.1 Swabs are moistened prior to the collection of samples using sterile Maximum Recovery Diluent (MRD) 0.1 % peptone & 0.85% NaCl for a minimum of five seconds.

4.1.2 The swabs are taken from the carcass sample sites by swabbing vertically, horizontally, and diagonally for not less than 20 seconds using a sterile 100cm^2 square template and as much pressure as possible.

Note : the surface area for swabbing is approximately 100cm^2 per sampling site; experienced personnel do not need to use a template.

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

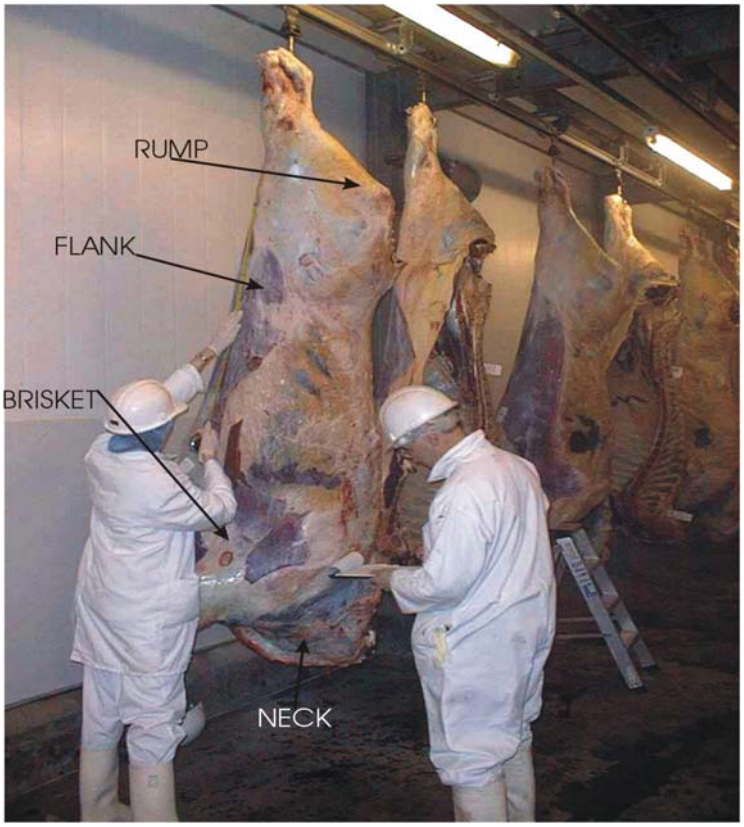


Diagram B: Recommended sampling sites for pigs

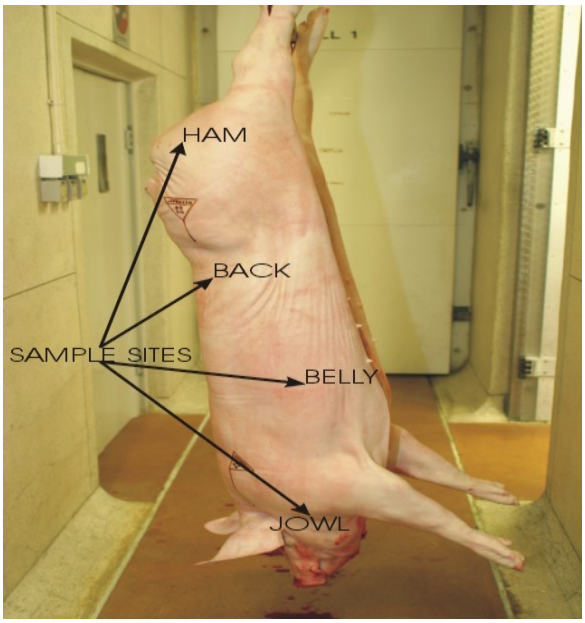
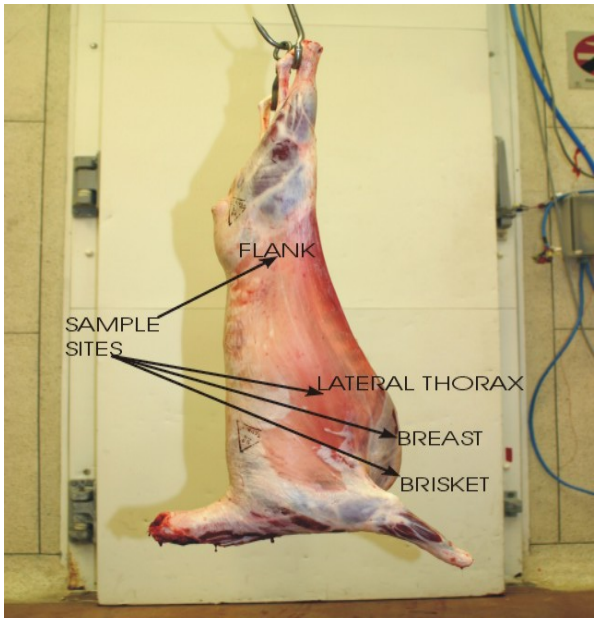


Diagram C: Recommended sampling sites for sheep



4.1.3 Sampling should be carried out after the completion of carcass dressing, but before the commencement of chilling.

4.1.4 A minimum of 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.

4.1.7 The 4 swabs from each carcass are then placed in a sterile container containing 100 mls of sterile diluent.

Note: it is imperative that the sample is taken under aseptic conditions and that the equipment used is sterile. Necessary sterility can be achieved by using:

- *Sterile cotton wool swabs*
- *Placing swabs in sterile, peristaltic type, homogeniser bags or sterile sample containers*
- *Sterile square templates of known internal areas*
- *Sterile disposable gloves*
- *Sterile scissors if used for cutting plastic swab sticks*
- *70 % Ethanol and portable gas blow torch or cigarette lighter*

or any other method which will achieve the same results

4.2 Sample maintenance, storage and transport

4.2.1 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.

4.2.2 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 the range of 15-300 cfu.

4.3.3 The analyses for *aerobic colony counts* 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 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/cm^2) for each pooled carcass sample (container) using the following formula:

$$\textbf{Cfu/cm}^2 = \frac{\textbf{Average cfu/plate x } \textit{a} \textbf{ (volume of original suspension)}}{\textit{b} \textbf{ (total surface area e.g. 100cm}^2 \textbf{ x 4 swabbing) x (dilution factor)}}$$

Example: Average cfu/plate = 140 on 10^{-2} dilution (Swabbing)

$$\text{cfu/cm}^2 = \frac{140 \times 100}{400 \times 10^{-2}}$$

$$= \frac{140 \times 100 \times 100}{400}$$

$$= \mathbf{3500\ cfu/cm^2}$$

4.3.5 The logarithm of each result is obtained by taking the logarithm to base 10 ($\log_{10}$) of cfu/cm^2 for each pooled sample from each carcass sampled on the day.

4.3.6 The mean log is calculated as the logarithm of the arithmetic average of all individual carcass counts in colony forming units per centimetre squared (cfu/cm^2) for all carcasses sampled on the day. An example of a calculation using data from five carcasses is as follows:

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 aerobic colony count falls within the acceptable range for cattle, 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/cm^2 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.

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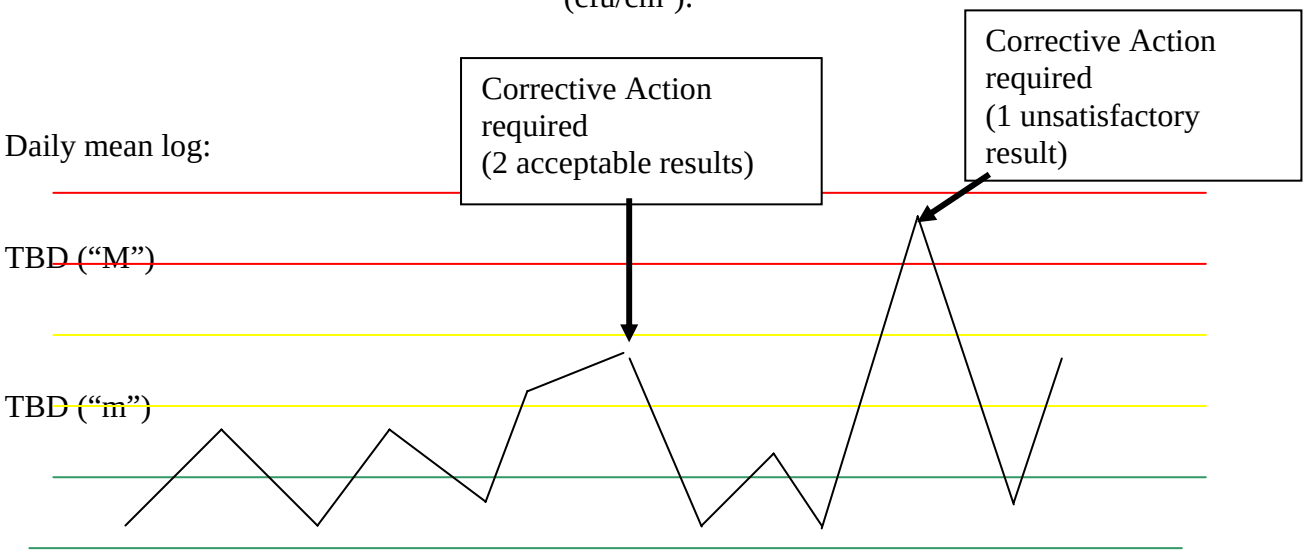
Presentation/evaluation of results and process control

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.

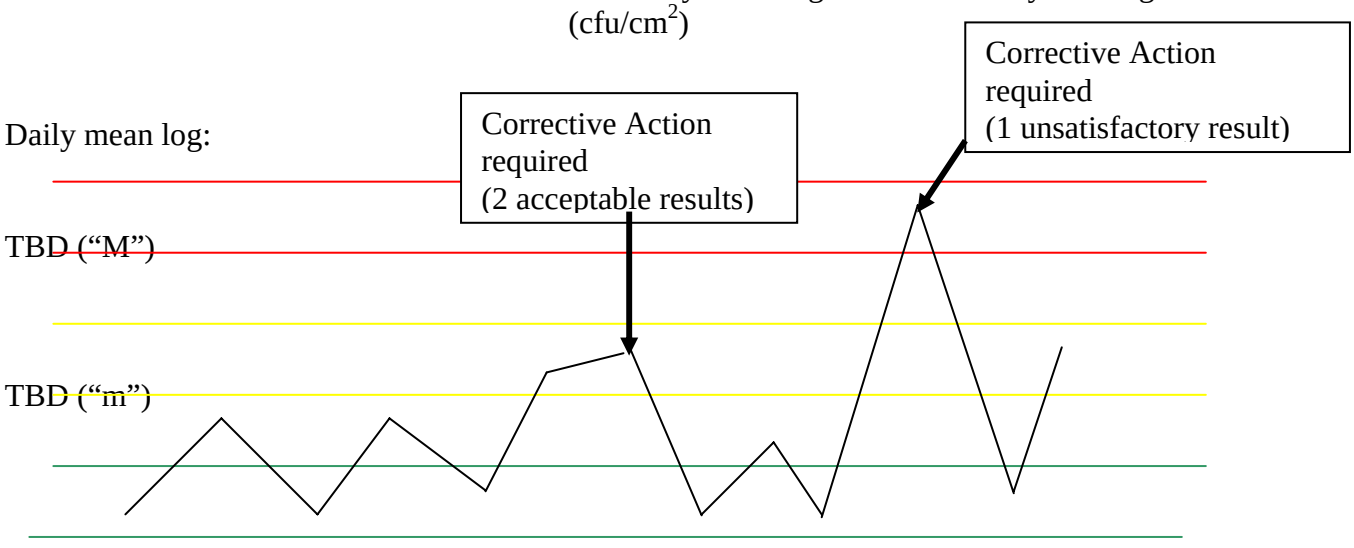
Process Control Chart A: Aerobic colony counts - daily mean log values of colony forming units (cfu/cm^2):



Comments:
Action taken:

No of weeks

Process Control Chart B: *Enterobacteriaceae*- daily mean log values of colony forming units (cfu/cm^2)



Comments:
Action taken:

No of weeks

TBD – To be determined by meat plant

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:

4 .4.2.1 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 responsible staff by the appropriate plant personnel.

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

4.4.2.2 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

Application of microbial criteria for this Process Control Chart are derived from criteria based on in company validated data from plant.

Daily mean log values (cfu/cm^2) for the wet/dry 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	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*
Enterobacteriaceae	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*	*TBD*

TBD – To be determined by meat plant

Note 1: In- company validation of microbial results to determine process control criteria may be carried out by a procedure based along the following guidelines:

1. *Sample the carcasses for ACC and* Enterobacteriaceae counts *using the excision SOP.*
2. *Sample the same carcasses using adjacent sites using the wet/dry procedure specified above.*
3. *Calculate the mean log as defined above for both excision and wet/dry swabbing.*
4. *The difference between the two results should then be evaluated statistically.*
5. *The validation outlined above can be completed in- house or by a competent agency approved by the Department of Agriculture and Food. The use of the final process control criteria is subject to the agreement of the official veterinarian.*

Note 2: The number of carcasses sampled should be sufficient to insure statistical validity (30 numerical results are acceptable for statistical evaluation).

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 Foods, 15th November 2005.