STANDARD OPERATING PROCEDURE FOR MICROBIOLOGICAL EXAMINATION OF CARCASSES BY THE ABRASIVE SPONGE SWABBING METHOD

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, pigs and sheep carcasses in slaughterhouses using the sponge (non-destructive) method.

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

An abrasive sponge technique is the only method permitted under regulation 2073/2005 for the analysis of Salmonella on carcasses. In this SOP carcasses are examined for aerobic colony counts (ACC), *Enterobacteriaceae* and Salmonella using an abrasive sponge technique.

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 log 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 Preparation of abrasive sponges

4.1.2 Two polyurethane sponges 10x10cm^2 are placed in each of two stomacher bags 20x30cm and moistened with 10mls of MRD (0.1 % peptone & 0.85% sodium chloride)

4.1.3 The bags are sealed and autoclaved at 121°C for 10 minutes.

Note: polyurethane *sponges are available from Ashtown Food Research Centre or alternatively these or the commercially available cellulose acetate sponge (speci-sponge) swabs may be purchased from the manufacturer preprepared.*

4.1.4 For composite/pooled samples two sponges are used as follows: using a sterile 100cm^2 square template and a sterile glove one sponge is taken and two sites swabbed using alternate sides and placed back in the original (first) bag. The second sponge is used to similarly swab the remaining two sites and this is placed back in the original (first) bag, which is sealed.

Note 1: 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 sponge swabs in sterile, peristaltic type, homogeniser bags or sterile sample containers*
- *Sterile square templates of 100cm^2*
- *Sterile disposable gloves*
- *70 % ethanol to sterilise square templates and portable gas blow torch or cigarette lighter*
  or any other method, which will achieve the same results.

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

Note 3: It is important to use firm and even pressure when taking the swab samples. Swabbing of each sample site (diagram D) a minimum of five times (in each direction) both vertically and horizontally is recommended.

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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

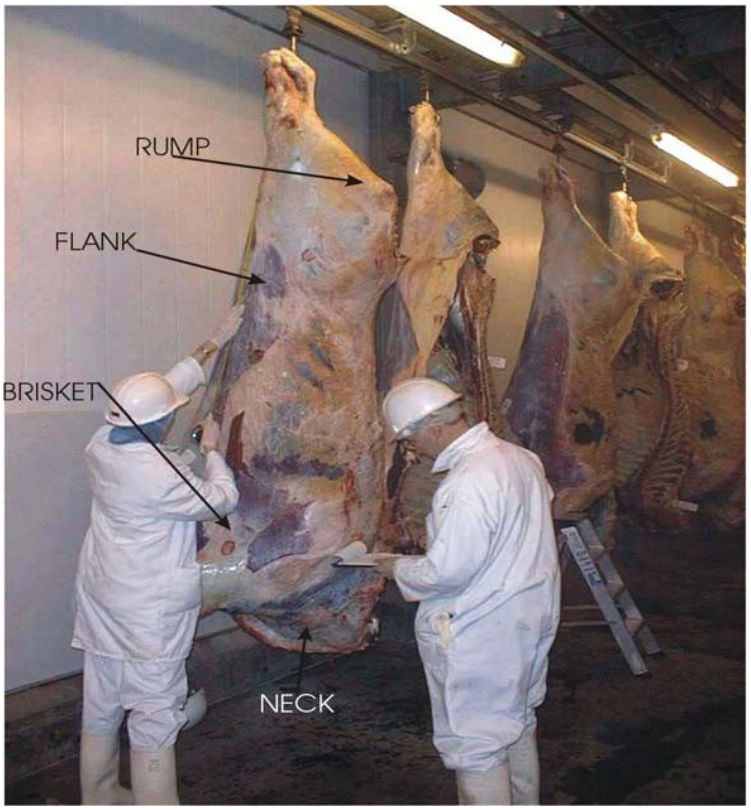

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Diagram B: Recommended sampling sites for pigs

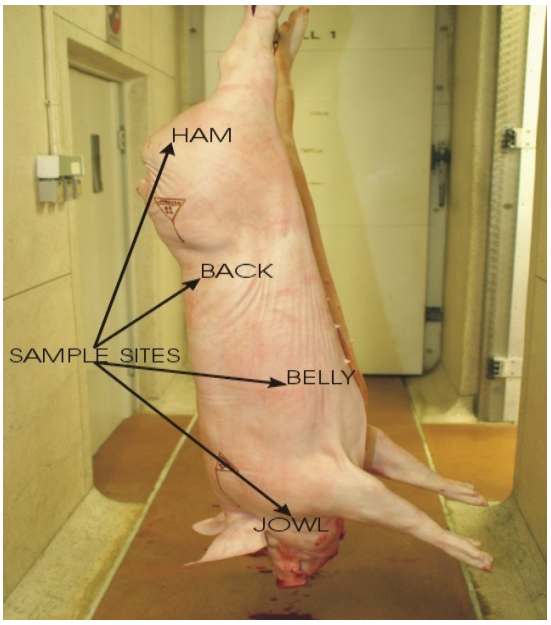

Diagram C: Recommended sampling sites for sheep

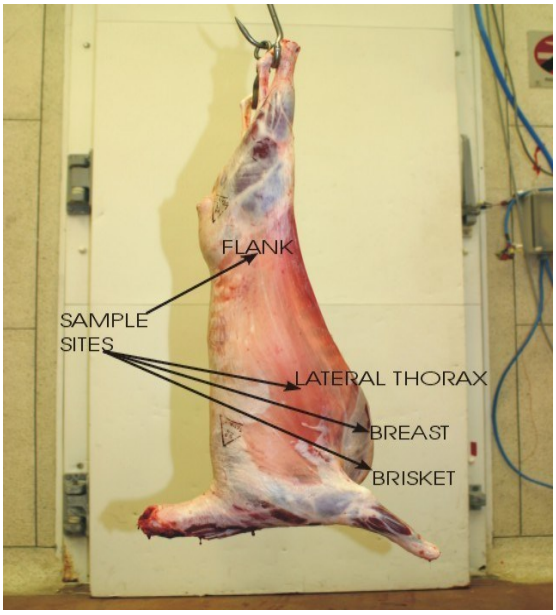

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Diagram D: Swabbing of carcass using polyurethane sponge

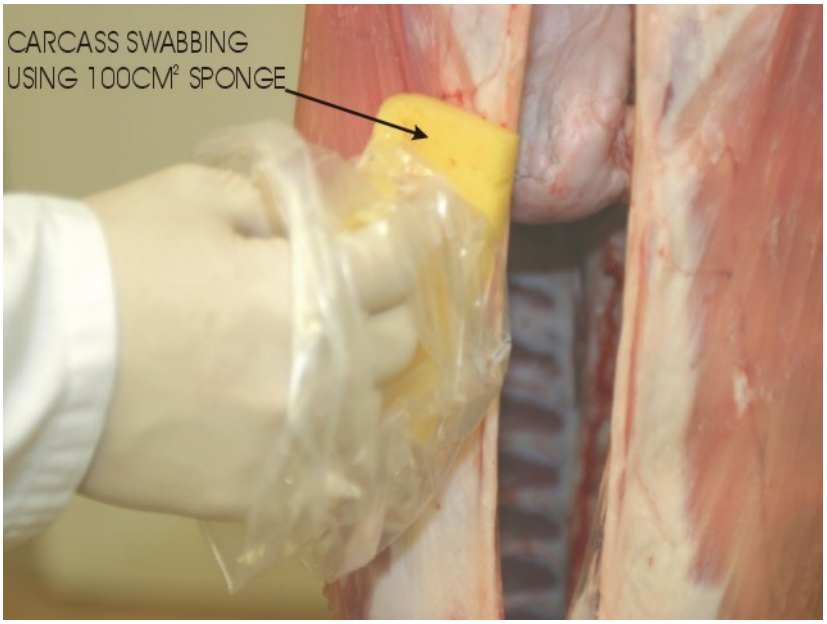
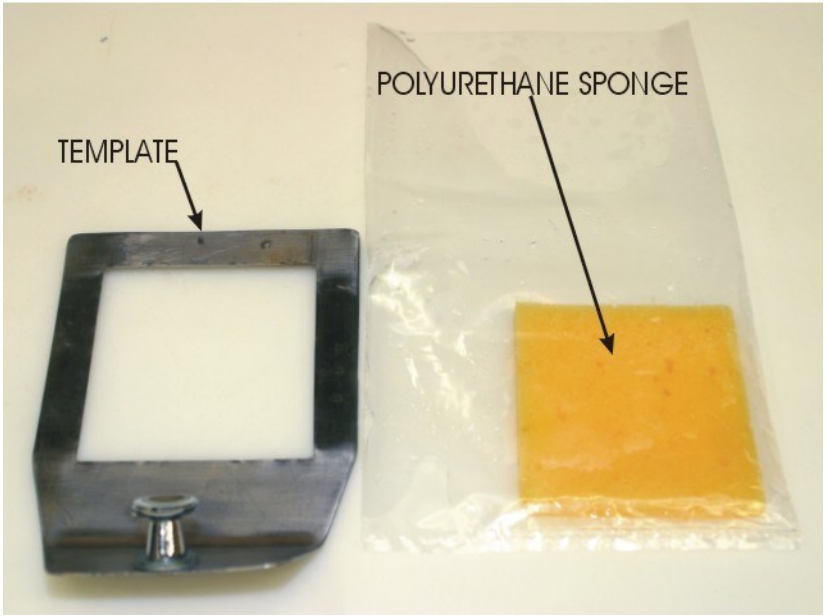


Diagram E: Template and sponge used to swab carcasses for bacteriological examination.



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

4.1.6 A minimum of 5 carcasses are sampled on one day of each working week.

4.1.7 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.

4.2 Sample maintenance, storage and transport

4.2.1 Each sample bag 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 bag 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: (Enumeration tests)

4.3.1 On receipt in the laboratory, 100ml of maximum recovery dilution medium (0.1% peptone, 0.85% sodium chloride solution) are added to the stomacher bag containing the two sponges and this is then homogenised using a peristaltic stomacher at a speed of 250 cycles/min for 2 minutes. This suspension in the stomacher bag is not a dilution and is represented in the calculation as the as the 10^0 dilution. This sample is then analysed for aerobic colony counts, *Enterobacteriaceae* and presence or absence of Salmonella.

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, *Enterobacteriaceae* and Salmonella 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: 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 For ACC and Enterobacteriaceae laboratory results are recorded as colony forming units (cfu/cm^2) for each pooled carcass sample (bag) using the following formula:

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

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 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. 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 ACC falls within the acceptable range for cattle, sheep.

Salmonella Presence or absence tests

4.3.7 The analysis for *Salmonella* Spp. is carried out using the following ISO procedure:
ISO 6579 'Horizontal Method for the detection of Salmonella species' From the initial suspension (10º) used for the enumeration tests, 25ml is transferred to 225ml of buffered peptone water and mixed well. The sample is then analysed in accordance with the procedure referenced above.

Laboratory reports

4.3.8 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. For ACC and *Enterobacteriaceae* the results 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. The salmonella results should be reported as presence/absence.

4.3.9 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.

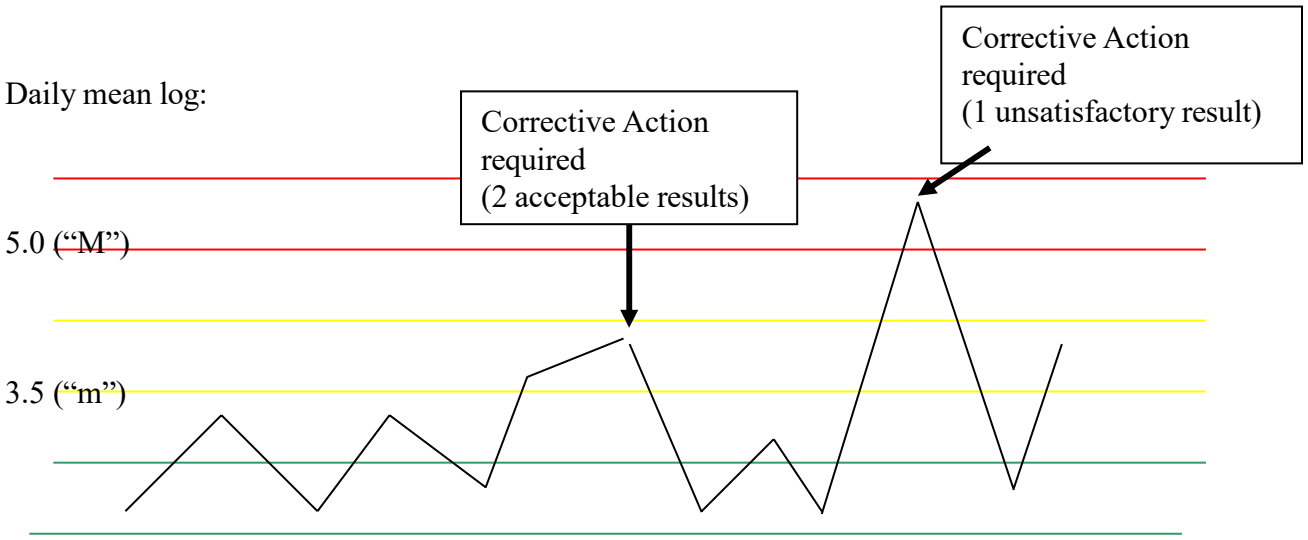
Presentation/evaluation of results and process control

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.

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

The process control chart for Salmonella in pig carcasses presents data for 10 consecutive weeks (50 samples in total) and shows the maximum number of samples (c) where Salmonella is permitted as 5.

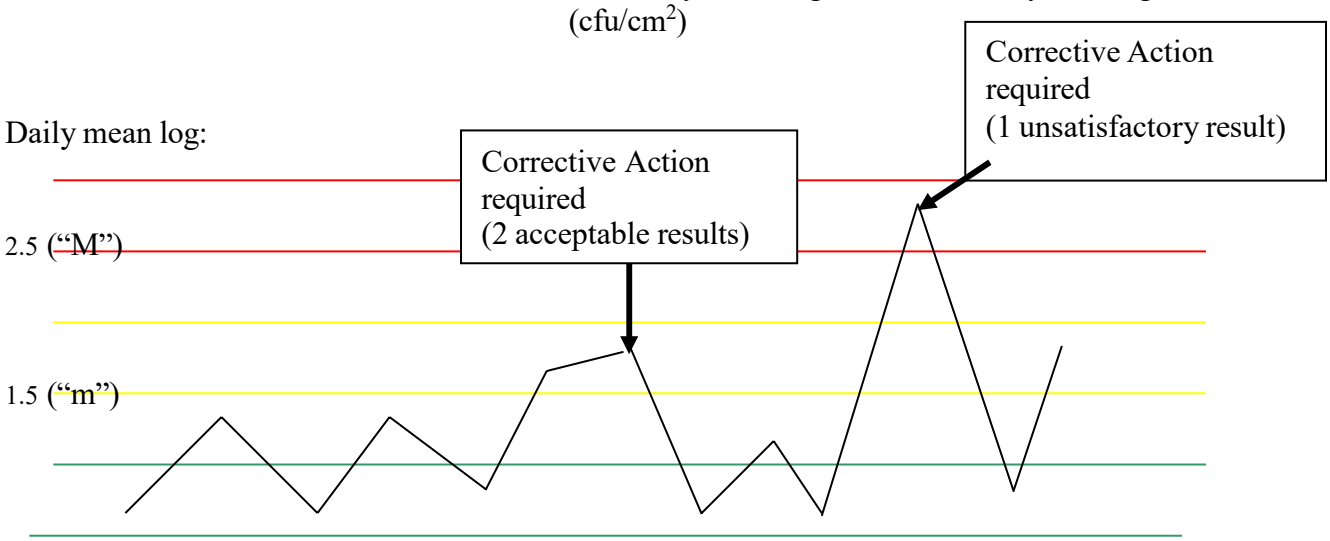
Process Control Chart A: Aerobic Colony counts- daily mean log values of colony forming units (cfu/cm^2):



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

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

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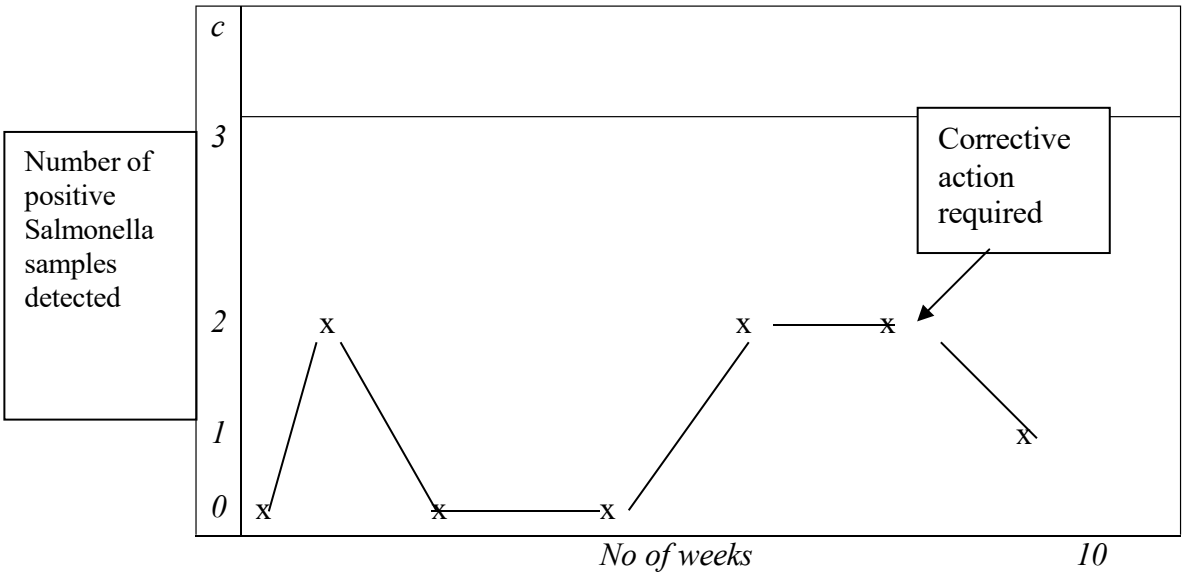
Process Control Chart C: Salmonella (pig carcasses)



Note 1: In the case of aerobic colony count and Enterobacteriaceae analysis the frequency of sampling may be reduced, to once per two week period, subject to the agreement of the official veterinarian, where, following six consecutive weeks of sampling, the average results are considered satisfactory. In the case of Salmonella analysis the frequency may be reduced to fortnightly, subject to the agreement of the official veterinarian, if satisfactory results have been obtained for 30 consecutive weeks.

Note 2: The day on which sampling is carried out is altered each week.

Note 3: Sampling frequency may be increased where the results of sampling over a six month period are unsatisfactory.

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

4.4.2 Corrective actions:

For ACC and *Enterobacteriaceae*, 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 *Salmonella* test results exceed the criteria laid down in the regulation corrective action must also be taken. This action is documented after consultation with the responsible staff by 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 and review of the origin of animals and of the biosecurity measures in the farms of origin

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

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

* Sponge swabbing versus excision method, equivalence data was validated using research conducted in the Food Safety Department at Ashtown Food Research Centre.

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Salmonella sampling plan using abrasive sponge technique:

Food category	Sampling plan		Limits
	n	c	
Carcasses of cattle, sheep, goats and horses	50*	2	Absence in the area tested per carcass
Carcasses of pig	50 *	3 **	Absence in the area tested per carcass

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.
* the 50 samples are derived from 10 consecutive sampling sessions.
** Updated in line with Commission Regulation (EU) No 217/2014

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'

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

Commission Regulation (EC) No 2073/2005, on Microbiological Criteria for Foodstuffs, 15th November 2005.

Commission Regulation (EU) No 217/2014, 7th March , 2014, amending Regulation (EC) No 2073/2005 as regards *Salmonella* in pig carcasses.

Gill, C.O. and Jones, T. (2000) Microbial sampling of carcasses by excision or swabbing. *Journal of Food Protection*, **63** (2), 167-173

Bolton, Sheridan and Doherty, (2000) HACCP for Beef Slaughter, ISBN 1 84170 121 1