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1. PURPOSE

To ensure that microbiological examinations of utensils, fittings and surfaces, carried out as part of a plant "own check" programme for the control of checks on cleaning and disinfection in meat establishments are carried out in accordance with the requirements of good manufacturing practice and Commission Regulation no. 2073/2005 on the microbiological criteria of foodstuffs.

2. SCOPE/RESPONSIBILITY:

Under Article 5 (2) of the regulation samples are required to be taken from processing areas and equipment used in food production, when such sampling is necessary in ensuring that criteria are met.

This standard operating procedure (SOP) is applicable to the microbiological examination of utensils, fittings and surfaces in slaughterhouses and cutting plants using swabbing and agar contact plate methods.

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

For the purpose of this SOP, analysis for aerobic colony counts (ACC) are used as indicators of surface hygiene.

3. **DEFINITIONS**

4. **PROCEDURE**

4.1 Sample sites

The food business operator should choose sample sites based on risk where it is desirable that the higher proportion of samples are derived from food contact surfaces.

The following points could be for example chosen:

Sterilisation devices for knives, junction of knife blade and handle, hollow blood draining knives, elastrators, scalding tanks, bung bagging machines, scraping/gambrelling table (pig). Sawblades and cutters, cattle dehiding, carcass dressing instruments, polishing machine, shackles and containers for transport, transport conveyor belts, aprons, cutting tables, flap doors

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if touched by passing carcasses chutes for food organs, parts of the line touched by carcases, overhead structures which may drip moisture etc.

In a poultry processing establishment the following sites could be chosen:

Sterilisation devices for knives, junction of knife blade and handle, scalding tanks, plucking machines, sawblades and cutters, evisceration equipment, containers for transport, transport conveyor belts, aprons, cutting tables, flap doors if touched by passing carcasses, chutes for food organs, parts of the line touched by carcasses, overhead structures which may drip moisture etc.

Note: the frequency of sampling should be a minimum of 10 samples or up to 30 samples in a large production area within a period of two weeks. If results are satisfactory over a period of time (2 months) the sampling frequency may be reduced following agreement with the official veterinarian. The places, which should receive the most attention, are the areas, which are destined to come in contact with the product. Roughly two thirds of the total number of samples should come from food contact surfaces.

To ensure all surfaces are tested in the course of a month a schedule should be made indicating which surfaces should be sampled and when. (See table A). The results must be monitored and recorded on charts to show the development over time (see 4.4.1 process control charts).

The following microbiological targets (ACC) for food contact and non contact surfaces may be used:

Satisfactory limit for	Unsatisfactory limit for
food contact surfaces	food contact surfaces
≤10	>10
Satisfactory limit	Unsatisfactory limit for
for non-food contact	non food contact
surfaces	surfaces
≤100	> 100

Note 1: Food contact surfaces should ideally be designed in a manner which facilitates thorough cleaning and disinfection e.g. stainless steel surfaces or high quality plastic material.

Note 2: The above limits are targets only and food business operators should strive to achieve more stringent targets where possible.

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TABLE A:

Example of sampling schedule

Sample sites, Week beginning:	Date/sampler/ time (nearest hour) of	ACC Results:		Corrective actions	Signed/ date
14/04/03	sampling	Satisfactory range (≤ 100) cfu/cm ² surface area, non food contact surfaces. (≤ 10) for food contact surfaces	Unsatisfactory range (> 100) cfu/cm ² surface area, non food contact surfaces. (> 10) for food contact surfaces		
Dehider	14/4 S. Smith 7.00 am	9		-	
Steriliser A- boning hall 1	14/4 S.Smith 7.00 am	2			
Steriliser B – slaughter line	14/4 S. Smith 8.00 am	4			
Knife blade/handle junction – boning hall 2	14/4 S.Smith 8.00 am		250	Informed cleaner, reviewed cleaning procedure, Will monitor closely on w/b 21/4	
Comments:					
Signed: superv	visor/manager	Da	ate:		

Note: It is desirable that surfaces of a nature which are easily cleaned for example flat stainless steel surfaces should aim for a target ACC of ≤ 10 .

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4.2 Sampling method

Agar plate contact method:

4.2.1 Agar plates (internal diameter 5cm) are prepared by pouring agar at 46°-48°C in a laminar flow cabinet until a convex surface is obtained.

Note: The prepared plates have a shelf life of one week at 2-4° C.

- 4.2.2 Any excess moisture on the surface of the prepared plates may be removed by inverting the plates in a 37°C incubator for approx 3hr.
- 4.2.3 Each plate is used by pressing onto a flat sampling site and incubating (see 4.4.5)

Swab technique:

- 4.2.4 Swabs are moistened prior to the collection of samples using 1ml of sterile maximum recovery dilution medium (0.1% peptone, 0.85% sodium chloride solution).
- 4.2.5 The swab is rubbed over a surface area of 20 cm^2 using a sterile template with firm pressure and rotation of the swab shaft using finger and thumb.
- 4.2.6 The sample area is swabbed horizontally, vertically and diagonally approx 10 times in each direction.

Note 1: for surfaces areas on which a template cannot be used, for example knife/handle junctions, the surface area of 20 cm^2 may be estimated by a skilled and trained operative.

Note 2: the template where used must be cleaned and sterilised after each use.

4.2.7 If equipment and surfaces are sampled following cleaning and disinfection, then a solution of 30g/l Tween 80 and 3 g/l lecithin (or other products with similar neutralising effect) should be added to the moistening solution for swabs.

Note: for wet areas, dry cotton swabs may be sufficient.

4.2.8 Swabs should be placed in a sterile container with 40ml maximum recovery dilution medium. This is the initial suspension.



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

- *1)* Sterile cotton wool swabs
- 2) Sterile square templates of known internal areas
- *3) Sterile disposable gloves*
- 4) Sterile scissors if used for cutting plastic swab sticks
- 5) 70 % ethanol and portable gas blow torch or cigarette lighter

or any other method, which will achieve the same results.

4.3 Sample maintenance, storage and transport

- 4.3.1 Each sample container should be identified with the sample site, time and date of sampling and the identity of the person who took the sample.
- 4.3.2 The sample container is stored between $0-4^{\circ}C$ (e.g.using a coolbox) and then transported to the laboratory under chilled conditions $0-4^{\circ}C$.

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

4.4 Laboratory examination:

- 4.4.1. The sample container should be shaken vigorously before serial dilution takes place.
- 4.4.2 Before plating, dilution should be carried out in 10 fold steps using the maximum recovery dilution medium (0.1% peptone, 0.85% sodium chloride solution)

Note: In preparing the dilutions 4ml is transferred from the 10^0 suspension into 36ml of diluent to give a dilution of 10^{-1} . This procedure is repeated with the further dilutions using a fresh sterile pipette for each decimal dilution.

Cfu/cm² = <u>Average cfu/plate x *a* (original suspension)</u> *b* x (dilution factor)

a is the volume of original suspension (e.g. 40) *b* is the total surface area (20cm²)

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4.4.3 The analyses for aerobic colony counts *should be* 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 $30 \,^{\circ}$ C.

Note 1: Methods other than the ISO method described above may be used where they have been approved by a recognised scientific body and 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.

- 4.4.4 Samples must be analysed for aerobic colony counts (ACC).
- 4.4.5 Inoculated agar plates and agar contact plates must be incubated for 24 hours at $30^{\circ}C$ +/- 1°C under aerobic conditions for ACC.
- 4.4.6 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, type, origin and identification of sample, date and hour of sampling and results as number of cfu per cm² of surface area.
- 4.4.7 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.

4.5 Presentation/evaluation of results and process control

4.5.1 Daily results are presented in the form of the following Process Control Charts (presented on a single sheet) that provide for ready identification of the need to take timely Corrective Action on the process. Records should be categorised as either acceptable of unsatisfactory for each site swabbed as indicated in the table below (see also table A, page 2 of 8).

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Process Control Chart A (non contact food surface): ACC- values of colony forming units (cfu/cm²)

Date:	Corrective Action required (1 unsatisfactory
/	result)
100	\
	V
0	
Comments:	
Action taken:	



Process Control Chart B (food contact surface): ACC - values of colony forming units (cfu/cm²)



Note: above plots are for illustrative purposes only

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- 4.6 Corrective actions:
- 4.6.1 Where a result exceeds the acceptable range, immediate corrective action is required to address the root cause of the problem. This action is documented after consultation with the responsible staff by the appropriate plant personnel.
- 4.6.2 Results in the unsatisfactory range are notified to the responsible staff as soon as possible. (see Process Control Chart above), including the individual responsible for the plant cleansing and sanitation programme.

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

5 **REFERENCES**

ISO 4833:2003, Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony-count technique at 30 $^{\circ}$ C.