

Department of Agriculture, Forestry and Fisheries

National Directorate Veterinary ~~Quarantine and~~ Public Health

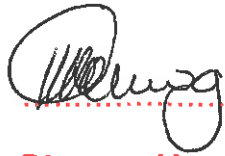
Notice No. VPN/15/2012-08	Date: 2012.08.24
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SUBJECT: Standard for the Microbiological Monitoring of Meat, Process Hygiene, and Cleaning and *Trichinella* Controls

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This VPN 15/2012-08 replaces VPN/15/2010-01.

It is effective as of the date of signature.



DR. K. MAGWEDERE

Director: Veterinary Public Health

30/01/2014

Date

PART I

DEFINITIONS

<i>Authorised person</i>	means any person authorised to exercise or perform any power or duty, or requested to render any service, by the Controlling Authority.
<i>Carcass</i>	means the whole body of a slaughtered animal after bleeding, evisceration and removal of the limbs at the carpus and tarsus, removal of the head, tail and the udder, and in addition, in the case of cloven-hoofed animals and solipeds, after flaying. However, in the case of pigs, removal of the limbs at the carpus and tarsus and removal of the head may be waived; In the case of ostriches carcass means the whole body of an ostrich after bleeding, plucking, flaying, evisceration, removal of the head and sectioning of the legs at the tibio-tarsometatarsal joint and the wings at the humero-radioulnar joint.
<i>Composite sample</i>	means samples from separate sources which are pooled for testing purposes.
<i>Controlling Authority</i>	means the authority which is directly responsible for the application of animal health or veterinary public health measures in a province of the country.
<i>Establishment</i>	means an approved slaughterhouse or an approved cutting plant or a unit grouping together several such establishments.
<i>Fresh meat</i>	means meat, including meat vacuum-wrapped or wrapped in a controlled atmosphere, which has not undergone any treatment other than cold treatment to ensure preservation.
<i>Meat</i>	means all parts of the carcass of bovine animals, sheep, goats, pigs, solipeds, ostriches, wild cloven-hoofed animals or wild solipeds.
<i>Official veterinary control</i>	means that the Controlling Authority knows the location of the animals and the identity of their owner or responsible keeper and is able to apply appropriate animal health measures, as required
<i>State Veterinarian</i>	A veterinarian authorised by the Controlling Authority to perform animal health and/or public health inspections.
<i>Batch of animals</i>	For the purposes of Trichinella controls a batch of animals will be regarded as that group of animals that derived from a single property in one consignment at the same time.

PART II

INTRODUCTION

1. Cognizance is taken of progressive developments in the European Union (EU) food safety legislation which prescribes certain microbiological standards for certain meats, meat preparations and meat products and monitoring of process hygiene, including microbiological monitoring of equipment and food contact surfaces.
2. Cognizance is further taken of the EU requirement that Food Business Operators (FBO) are obliged to comply with the microbiological standards for meat and meat preparations/products, process hygiene and process environments by implementing comprehensive microbiological testing programmes.
3. It is further noted that South Africa currently has no equivalent legislation and therefore needs to prescribe additional procedures applicable to export establishments approved to export to the EU, to ensure equivalent outcomes and standardization of such microbiological testing programmes. Export certification to the EU will only be permissible where compliance to EU requirements can be demonstrated.
4. Abattoirs and cutting plants implement monitoring programs and documented systems whereby the effectiveness of measures to control the hygiene of production can be validated and verified. Factors that have the potential to adversely affect the safety of meat are rigorously monitored and controlled. The microbiological status of meat is used as an indicator of the adequacy of process interventions and process hygiene. However, these programs are only as valid as the competency and reliability of the laboratory performing the analyses.
5. A Laboratory Approval Program was designed to provide a credible, independent system to verify that laboratories are competent to carry out tests required to verify hygiene of production **and for *Trichinella* examination**.
6. The procedures adopted by the Department of Agriculture, Forestry and Fisheries encompass all aspects of a microbiological monitoring program, including the development of standardised sampling plans, sampling and transportation procedures and analytical methods and the verification of laboratory proficiency.

PART III

RESPONSIBILITIES

1. MANAGEMENT OF THE ESTABLISHMENT

The management of the establishment will be responsible for:

- 1.1 Initiating microbiological testing of dressed carcasses, primal meat cuts, retail meat portions and working surfaces in accordance with this Veterinary Procedural Notice and as dictated by the State Veterinarian.
- 1.2 Keeping microbiological testing results as prescribed in Annex 3 of this VPN as part of the Hygiene Management System.
- 1.3 Making microbiological test results available to the State Veterinarian.
- 1.4 Meeting all costs in this respect.
- 1.5 Performing company initiated corrective actions as well as corrective actions as dictated by the State Veterinarian where unsatisfactory results were obtained.

2. CONTROLLING AUTHORITY

The State Veterinarian must:

- 2.1 Review all microbiological testing results and take these into account when evaluating the efficacy of the Hygiene Management System at the establishment.
- 2.2 Inform the management of the establishment and the Controlling Authority of any negative trends.
- 2.3 Require extra sampling if deemed necessary.
- 2.4 Collect official control samples every 3 months and submit the sample to officially approved laboratory. The results of these tests must verify the results obtained by the FBO.
- 2.5 Investigate instances where unsatisfactory results were obtained.
- 2.6 Monitor the effectiveness of corrective actions and take legal recourse if necessary.

PART IV

MICROBIOLOGICAL TESTING PROGRAM

1. Maximum allowable levels

1.1 Maximum allowable meat microbiological levels

See Annex 1 attached hereto.

1.2 Mean values for the number of colonies on work surfaces

See Annex 2 attached hereto.

2. PRODUCTS TO BE SAMPLED

2.1 Carcasses (post slaughter and dressing)

Samples are to be collected within 30 minutes after inspection.

2.2 Primal cuts (bone out) and retail packed meat

The boning of primal cuts is considered to be complete immediately prior to secondary chilling or freezing.

Time of sampling:

- a. Primal cuts: Immediately prior to vacuum packaging, wrapping or bulk packing into cartons.
- b. Retail packed meat: Immediately prior to closing and sealing of vacuum packaging.

3. FREQUENCY OF SAMPLING

The following samples must be collected at least once a week or every five working days⁽³⁾:

Products to be sampled	No. of units to be sampled	No. of sites per unit to be sampled.	Location of sample sites	Sample size/sample site	Pooled sample	Single sample	Pooled sample size	Part of process to be used for sample collection	Test for:	Total number of samples to be collect per week
Carcasses ⁽¹⁾	5 carcasses ⁽²⁾	4	See Annexures 6 - 8	5 cm ²	Yes	X	20 cm ²	Within 30 minutes after slaughter	TVC. Enterobacteriaceae, E.coli.	5x20 cm ²
Carcasses ⁽¹⁾	5 carcasses ⁽²⁾	4	See Annexures 6 - 8	6.25g	Yes	X	25g	Within 30 minutes after slaughter	Salmonella	5x25g
Primal meat cuts ⁽¹⁾	4 cuts ⁽²⁾	1	Not prescribed	5 cm ²	Yes	X	20 cm ²	At the point immediately prior to plastic wrapping	TVC. Enterobacteriaceae, E.coli.	1x20 cm ²
Primal meat cuts ⁽¹⁾	4 cuts ⁽²⁾	1	Not prescribed	6.25g	Yes	X	25g	At the point immediately prior to plastic wrapping	Salmonella	1x25g
Retail portions ⁽¹⁾	4 cuts ⁽²⁾	1	Not prescribed	5 cm ²	Yes	X	20 cm ²	At the point immediately prior to plastic wrapping	TVC. Enterobacteriaceae, E.coli.	1x20 cm ²
Retail portions ⁽¹⁾	4 cuts ⁽²⁾	1	Not prescribed	6.25g	Yes	X	25g	At the point immediately prior to plastic wrapping	Salmonella	1x25g

- (1) Beef, sheep, goats, horses, wild cloven hoofed game, wild solipeds, ostriches. Where more than one species are slaughtered/processed per week one of each species must be done per week on a rotational basis to ensure that equal sampling is done from each species.
- (2) Randomly selected.
- (3) The total number of samples collected per week is seven samples for Total Viable Count and Enterobacteriaceae and another seven samples for Salmonella, 14 samples in total.

4. EVALUATION OF RESULTS

- 4.1 Evaluation of results is based on a three class outcome system as described in Annex 1. Performance of the sampling plans is defined by parameters, **m**, **M**, **n**, and **c**, where:

'm' is a defined value separating a good result from a marginally acceptable result (values between **m** and **M** are considered to be marginally acceptable)

'M' is the maximum value for a marginal result (values greater than **M** are unacceptable)

'n' is the number of individual samples in a sampling plan (also called a sampling window)

'c' is the number of marginal samples allowed in '**n**' samples

- 4.2 Based on these parameters a sample result can fall into one of three classes:

Acceptable: less or equal to the defined limit (**m**) ($\leq m$ cfu/cm²)

Marginal: greater than **m** but not higher than **M** ($>m$, but $\leq M$ cfu/cm²)

Unacceptable: results greater than **M** ($>M$ cfu/cm²)

- 4.3 Assessment is by means of a moving window which is regarded as the last sample result and those preceding it up to the value of **n**. As new sample results become available this will cause the window to move on including the new samples as part of the sampling plan or window (**n**) assessment. To allow for corrections in the process to be evaluated a window will be reset after each failure and subsequent corrective actions. Please refer to Annex 3 for an example and Annex 4 for further explanation of the principle.
- 4.4 When a sample window fails the FBO must immediately inform the State Veterinarian. The establishment will also initiate an investigation into the causative factors and implement the necessary corrective and preventative actions. This must be recorded in the official Corrective Action Procedures Programme at the establishment.
- 4.5 Where a sampling plan/ window have failed export certification may be withheld by the State Veterinarian until corrective actions have been completed to his/her satisfaction. The meat safety risk involved as well as the extent of the investigation, the corrective actions implemented by the Food Business Operator and possible pattern of recurrence of unacceptable results will be the determining factors in making the decision.
- 4.6 The Food Business Operator must, in addition to recording of results in the format prescribed in Annex 3 of the VPN, also depict the average of all microbiological sample results for Total Viable Counts obtained for each week, graphically, where average results are plotted against every week of the year.

PART IV

SAMPLING, TESTING METHODS AND TRANSPORTATION

1. Equipment and instruments

1.1 Method (Carcasses, primal meat cuts and retail portions)

1.1.1 Cooler bag (container)

Appropriate size cooler bag (container) and sufficient ice in plastic bags. Good quality insulated cooler containers (polystyrene or other types) are efficient. As a further measure to keep samples as close as possible to 0°C, ice in waterproof plastic bags can be layered inside the container and the samples placed between the layers. Samples must reach the laboratory before the temperature rises above 4°C.

1.1.2 Sample bags

Stomacher bags (80 ml or 400 ml) or other sterile plastic bags are recommended.

1.1.3 Alcohol

A jar with a wide mouth containing 70% alcohol in which the cork cutter "heads" (or plug bore tips) are kept. During sampling the handles are also put into the alcohol.

A flask or bottle with 70% alcohol to be used for wiping the plastic cover of the products in the case of sampling of vacuum-wrapped product.

1.1.4 Cotton wool

Cotton wool to be used for cleaning the plastic surface with 70% alcohol in the case of sampling of vacuum-wrapped product.

1.1.5 Elastic bands

After the plastic bags with the samples are properly marked and folded several times to make it tight, they are secured in a tight folded position with elastic bands.

1.1.6 Scalpel

A scalpel with disposable blades to separate disks of meat cut by the cork cutter, if samples are taken from carcasses, or to cut a triangle (two sides of it) into the plastic wrapping, if wrapped product is sampled.

The bigger size scalpel is more convenient to use. Provide for a new disposable and sterile blade for every 2-3 sets of samples. Between taking the different sets of samples, the blade must be sterilized.

1.1.7 Forceps

A standard forceps, ± 125 mm length. (A rat tooth forceps can sometimes be troublesome when loose connective tissue is present.)

1.1.8 Cork cutter

Cork cutters can be used for taking samples of meat, in chilled or frozen form. (A plug bore may also be used for frozen samples and is recommended as it could be quite difficult to take these samples with a cork cutter.)

A cork cutter with an inner diameter of 25,2 mm and a surface area of 5 cm². It is prescribed that the above size of the cork cutter is used to standardize sampling equipment.

1.1.9 (Methylated) spirit-lamp

To sterilize the cork cutter, forceps and scalpel-blade. These are put into 70% alcohol and flamed in the spirits burner.

1.1.10 Pencil

Pencil with permanent (indelible) ink for marking of the plastic sample bags.

Note: Always record

- a. the time (hour and minutes) of sampling,
- b. the date of sampling,
- c. farm of origin,
- d. temperature at sampling
- e. number of pieces or surface area sampled (in case of composite samples) and
- f. the product sampled.

Correct forms for the laboratory must be obtained before hand. The collection of the samples should be done with the necessary precautions as far as sterility is concerned and samples should be kept on ice till delivered to the laboratory.

Arrangements with the laboratory prior to the taking of the samples should be done as to confirm the logistics and correct laboratory techniques used.

1.2.1 Equipment required are:

Cooler bag (container) and sufficient ice in plastic bags
 Sample bags
 Alcohol
 Elastic bands
 (Methylated) spirits-lamp
 Pencil for marking sample bags.

2. Composite sample

2.1 Composite samples in a sterile bag and neatly closed should weigh \pm 20 - 25 grams each.

The total number of grams per pooled sample must always be > 25 g for the analyses for Salmonella, which is usually required a zero status, i.e. present or absent result.

3. Sampling Methods

3.1 Sampling Method (Carcasses, primal meat cuts and retail portions)

- a. Put cork cutter heads and handles (or plug bore tip), scalpel and forceps in 70% alcohol.
- b. Mark sample bags.
- c. Flame instruments before use and between bags.
- d. Cut out samples and put in sample bags to make up composite samples.
- e. Fold sample bags and secure with elastic bands.
- f. Store between layers of ice in cooler bag (container).

4. Transportation

4.1 Temperatures as close as possible to 0°C, e.g. when samples are kept between layers of ice in an insulated container, is the most reliable and safe and samples can be kept at this temperature for ± 12 hours without seriously affecting the growth of the organisms to be analysed.

4.2 Care must be taken that the temperature of the container in which the samples are kept, is not allowed to go above 20°C at all!

NB: Samples taken of chilled meat should never be frozen. Samples taken from frozen product is at $\pm -18^\circ\text{C}$ and therefore must be kept frozen.

Very low temperatures, lower than -15°C , can kill off between 10-50% of the aerobic bacteria, although less harm is done to organisms like Salmonellae, Escherichiae and other spore organisms in general, than to aerobic bacteria.

5. Laboratory techniques

- 5.1 Samples must be of known surface to be able to report the microbial count as number of organisms per cm².
- 5.2 Most of the bacteria on the product are actively bonded to the tissues. To be able to obtain reliable results, it is therefore necessary to have the sample macerated in a stomacher (a total destruction technique). It should be done after weighing the sample and adding a suitable diluent to the sample.
- 5.3 To be able to determine accurate results, serial dilutions should be made up to a 10⁴ dilution.
- 5.4 Only the methods prescribed in Annex 1 of this VPN may be used for analysis, except if prior permission had been obtained from the Controlling Authority to use a different method with equivalent outcome.

6. Laboratory report

- 6.1 The laboratory report should contain the following details:
 - a. Time and date of receipt of the sample at the laboratory and temperature of sample.
 - b. Proper identification of the sample especially pertaining to the point of collection.
 - c. Confirmation that the prescribed collection method was followed in the collection of the sample. (If the sample is collected by personnel of the laboratory doing the analysis.)
 - d. Confirmation that the prescribed transport steps were followed.
 - e. Confirmation that the correct handling procedures were followed at the laboratory.
 - f. Date and time of analysis at the laboratory.
 - g. Time of reading results.
 - h. Results of the analysis.
 - i. Range of criteria for evaluation (See Annex 1 of this VPN).

7. Handling of samples at the laboratory

- 7.1 Study all records and correspondence related to the required sample and ensure that all the relevant information has been supplied and is legible.

Important: Record the temperature of the sample and time of arrival and include it in the laboratory report.

- 7.2 It is important that the analyst has a thorough knowledge of the physical characteristics of both the normal and the abnormal products, either by techniques or experience.

PART VI

LABORATORIES

1. Laboratory Approval Program

- 1.1 Laboratories performing microbiological analyses and examination for *Trichinella* for establishments approved to export fresh meat from the Republic of South Africa must take part in the Laboratory Approval Program.
- 1.2 The Laboratory Approval Program is managed by Me Lizzy Molele at the Directorate: Veterinary ~~Quarantine and~~ Public Health in Pretoria. Her contact details are as follows:

The Director
Directorate: Veterinary ~~Quarantine and~~ Public Health
Private Bag X 138
Pretoria
0001
Tel: 012-319 7501
Fax: 012- 319 7699
E-Mail: LizzyM@daff.gov.za
For attention: Me. L. Molele

PART VII

TRICHINELLA CONTROLS

The export of wild soliped meat (zebra) and crocodile meat is subjected to the controls described in this section. The controls are implemented on the basis of Commission Regulation (EC) No. 2075/2005 as amended, laying down specific rules on official controls for *Trichinella* in meat, the export of wild soliped meat (zebra) and crocodile meat.

1. Because no *Trichinella* has ever been detected in pigs, in farmed crocodiles or in zebra meat tested for export to the European Union (EU), traceability regarding *Trichinella* examination results will be acceptable if traceability of any suspect or positive sample is traceable to the batch of animals slaughtered and deboned at an export establishment. It must be noted however that in cases where traceability is only possible onto batch level, rather than to an individual carcass, any positive results will result in condemnation, for human consumption, of all meat that comprises the particular batch and in cases of suspect results, will result in refusal of all the meat that comprises the particular batch for export certification. Where samples are however traceable to individual carcasses, only individual infested or suspect carcasses will be either condemned for human consumption or refused an export approval mark, depending on whether the results are positive or suspect.
2. The following samples for *Trichinella* examination must be collected from each individual carcass as part of the post-mortem inspection procedure and examined as pooled samples:

Specie	Muscle sample site	Muscle sample size/carcass	Pool size for testing
Zebra	Lingual or Masseter or if these are not available, Diaphragmatic pillar	≥ 5g	20 carcasses x 5g/carcass = 100g
Crocodile	Pterygoid and/or Masseter and/or Intercostal	≥5g	20 carcasses x 5g/carcass = 100g

3. Meat samples may only be examined by a laboratory that has been approved by the Department of Agriculture, Forestry and Fisheries (DAFF), specifically for doing *Trichinella* examinations. The laboratory shall inform the official veterinarian in charge of the establishment of positive results as soon as they are known. Deltamune is currently the only laboratory approved by DAFF and all samples must be analysed by this laboratory. The cost of sample analysis is payable by the export establishment or exporter. Deltamune has been approved by DAFF for the magnetic stirrer method of pooled sample digestion as prescribed in Chapter 1 of Annex 1 of Commission Regulation 2075/2005.
4. Unless a specific Hygiene Management Programme (HMP) to prevent dispatch of export of unapproved meat from the premises has been compiled by the Food Business Operator (FBO) and approved by the Official Veterinary Inspector (OVI), export approval marks may only be applied to packaging containing cuts of export

meat once the results of the *Trichinella* examination have been received and if the particular batch of meat tested negative for *Trichinella*. The aim of the HMP to prevent dispatch or export of unapproved meat from the premises/export establishment is to keep all batches of meat for which *Trichinella* examination results are pending in bond, until satisfactory results have been received.

5. The FBO must compile a contingency plan that will indicate what steps will be taken in case positive *Trichinella* results are received for a particular batch of meat. This must include, but is not limited to:
 - Reassessment of the establishment's HACCP system and plans.
 - Identification, tracing and detention of all the meat that comprises this specific batch of meat that tested positive for *Trichinella*.
 - No batches of meat, where a positive *Trichinella* result has been received or where a suspect result for *Trichinella* has been received, may be certified for export, unless positive results are traceable to meat from individual, infested carcasses.
 - Batches of meat where a positive *Trichinella* result has been received, must be condemned for human consumption, unless the infestation is traced to a single carcass, in which case only that particular carcass and all the meat derived from it, must be condemned for human consumption and the rest of the batch will be suitable, both for human consumption and export certification.
 - The *Trichinella* nematode that was detected must be submitted to Deltamune for species identification.
 - The state veterinarian responsible for the area where the infested animals originated, as well as the Director: Animal Health must be informed of the infestation.
6. No export certification may be issued by the OVI, unless the requirements of this section, read in conjunction with Commission Regulation (EC) 2075/2005 have been met.

Annex 1: Microbiological standards for export meat

Category	Micro-organisms	Sampling Plan		Limits		Method
		n ⁽¹⁾	c ⁽²⁾	m ⁽³⁾ (log value)	M ⁽⁴⁾ (log value)	
Carcasses and meat cuts of cattle, sheep, goats and horses	Aerobic colony count	35	7	3162 cfu/cm ² (3.5 log)	100 000cfu/cm ² (5.0 log)	ISO 4833
	Enterobacteriaceae	35	7	31 cfu/cm ² (1.5 log)	316 cfu/cm ² (2.5 log)	ISO 21528-2
	<i>E.coli</i>	35	7	1 cfu/cm ² (0 log)	10 cfu/cm ² (1 log)	
	<i>Salmonella</i>	50	2	Absent in area tested per carcass	Absent in area tested per carcass	EN/ISO 6579
Carcasses and meat cuts of wild cloven hoofed game and wild solipeds	Aerobic colony count	35	7	100 000cfu/cm ² (5.0 log)	550 000cfu/cm ² (5.7 log)	ISO 4833
	Enterobacteriaceae	35	11	100 cfu/cm ² (2.0 log)	316 cfu/cm ² (2.5 log)	ISO 21528-2
	<i>E.coli</i>	35	11	50 cfu/cm ² (1.7 log)	500 cfu/cm ² (2.7 log)	
	<i>Salmonella</i>	50	2	Absent in area tested per carcass	Absent in area tested per carcass	EN/ISO 6579
Carcasses and meat cuts of ratites	Aerobic colony count	35	7	3162 cfu/cm ² (3.5 log)	100 000cfu/cm ² (5.0 log)	ISO 4833
	Enterobacteriaceae	35	7	31 cfu/cm ² (1.5 log)	316 cfu/cm ² (2.5 log)	ISO 21528-2
	<i>E.coli</i>	35	7	1 cfu/cm ² (0 log)	100 cfu/cm ² (2 log)	
	<i>Salmonella</i>	50	2	Absent in area tested per carcass	Absent in area tested per carcass	EN/ISO 6579

(1) 'n' is the number of individual samples in a sampling plan (also called a sampling window)

(2) 'c' is the number of marginal samples allowed in 'n' samples

(3) 'm' is a defined value separating a good result from a marginally acceptable result (values between m and M are considered to be marginally acceptable)

(4) 'M' is the maximum value for a marginal result (values greater than M are unacceptable)

Annex 2: BACTERIOLOGICAL SAMPLING FOR CHECKS OF CLEANING AND DISINFECTION IN SLAUGHTERHOUSES AND CUTTING PLANTS

Standard for the microbiological monitoring of meat, process hygiene, and-cleaning and Trichinella Controls Revision 24/08/2012

The described bacteriological sampling should be applied according to sanitation standard operating procedures (SSOPs) specifying the pre-operational hygiene controls to be carried out in areas which have a direct bearing to product hygiene.

SAMPLING METHOD

This Annex describes the contact plate method and the swab technique. The use of these methods is limited to the testing of surfaces, which are cleaned and disinfected, dry, flat, sufficiently large, smooth.

They should always be used before production starts — never during production. If visible dirt is present cleaning should be judged as unacceptable without any further microbiological evaluation.

This method is not suitable for sampling meat or meat products.

Methods offering equivalent guarantees may be used after approval by the controlling authority.

AGAR CONTACT PLATE METHOD

For the agar contact plate method, small plastic dishes with lids (i.e. internal diameter 5,0 cm) filled with plate count agar (according to ISO, actual version) and dishes filled with violet red bile glucose agar (VRBG agar according to ISO, actual version) are pressed on to each sampling site and subsequently incubated. The contact surface of each plate is 20 cm².

After preparation the agar has a shelf life of approximately three months when kept at 2 to 4 °C in closed bottles. Shortly before preparation of the plates, the relevant agar has to be melted to 100 °C and cooled to 46 to 48 °C. The plates have to be placed in a laminar air flow cabin and should be filled with agar until a convex surface is obtained. The prepared plates should be dried before use by incubating them upside down overnight at 37° C. This is also a useful check for possible contamination during preparation; plates with visible colonies must be discarded.

The plates have a shelf life of one week at 2 to 4 °C, when sealed into plastic bags.

SWAB TECHNIQUE

Samples should be collected with cotton swabs moistened with 1 ml of 0,1 % NaCl peptone solution (8,5 g NaCl, 1 g trypton casein-pepton, 0,1 % agar, and 1 000 ml distilled water) from a surface area of preferably 20 cm² marked with sterile template. If sampling is performed following cleaning and disinfection an amount of 30g/l Tween 80 and 3g/l Lecithin (or other products with a similar effect) should be added to the moistening solution for swabs. For wet areas dry cotton swabs may be sufficient.

The swabs should be held in sterile forceps and the sampled surface must be swabbed 10 times from top to bottom applying a firm pressure on the surface. Swabs should be collected in a bottle containing 40 ml buffered peptone with 0,1 % agar saline solution. The swab samples must be refrigerated at 4 °C until further processing. The bottle should be shaken vigorously before diluting in 10-fold steps in 40 ml 0,1 % NaCl peptone solution followed by microbiological examination (e.g. drop-plating technique).

FREQUENCY

Always, a minimum of 10 samples or up to 30 samples in a large production area should be carried out within a period of two weeks. Three samples should be taken from large objects. If the results are satisfactory over a period of time the frequency of sampling may be reduced following the agreement of the official veterinarian. Places which should receive most attention are the areas which are destined to come or may come into contact with the product. Approximately two thirds of the total number of samples should be taken from food contact surfaces.

To ensure that all surfaces are tested in the course of a month, a schedule should be made indicating which surfaces should be sampled on which days. The results must be recorded and regular bar charts are to be made to show the developments with time.

TRANSPORT

The used contact plates do not need to be cooled during transport and before incubation. Swab samples must be cooled until further processing to 4 °C.

BACTERIOLOGICAL PROCEDURES

In addition to the given description, ISO-methods may be used.

The bacterial counts should be reported according to the number of organisms per cm² of surface area. Inoculated plate count agar plates and agar contact plates must be incubated for 24 hours at 37 °C ± 1 °C under aerobic conditions for total colony count (TVC). This procedure must take place within two hours of sampling. The number of bacterial colonies should be counted and recorded.

For quantitative estimation of Enterobacteriaceae VRBG agar must be used. Incubation of inoculated plates and agar contact plates must begin within two hours of sampling under aerobic conditions. After 24 h incubation at 37 °C ± 1 °C, the plates must be examined for Enterobacteriaceae growth.

Analysis should be performed for total viable counts.

SAMPLING SITES

The following points should, for example, be chosen as sampling sites: sterilisation devices for knives, knives (junction of blade and handle), hollow blood draining knives, elastrators, scalding tanks, bung bagging machines, scraping/gambrelling table (pig), sawblades and cutters, cattle dehiding, other carcase dressing instruments, shackles and containers for transport, transport conveyor belts, aprons, cutting tables, flap doors if touched by passing carcasses, chutes for food organs, parts of the line often touched by carcasses, overhead structures which may drip moisture, etc.

CALCULATING THE RESULTS

For the agar contact plates and for the TVC and Enterobacteriaceae counts of the swab tests, the results have to be entered on a registration form. For the purpose of process control verification of cleaning and disinfection only two categories for TVC and Enterobacteriaceae have been established: acceptable and unacceptable. The acceptable range for the number of colonies on an agar contact plate and the number of colonies of TVC or Enterobacteriaceae (results from swab tests) are shown in table 1.

Table 1:

Mean values for the number of colonies for testing of surfaces

	Acceptable range	Unacceptable
Total viable counts (TVC)	0-10/cm ²	> 10/cm ²
Enterobacteriaceae	0-1/cm ²	> 1/cm ²

FEEDBACK

The results of the test have to be reported to the responsible staff as soon as possible. The results should be used to maintain and improve the standard of cleaning and disinfection. Causes of unsatisfactory results should be clarified by consultation with the cleaning staff. The following factors may be involved: 1. absence or inadequacy of training and/or instructions, 2. the use of unsuitable cleaning and/or disinfection materials and chemicals, 3. inadequate maintenance of cleaning apparatus, and 4. inadequate supervision.

**Annex 4: EXAMPLE OF COMPLETION AND EVALUATION OF
MICROBIOLOGICAL RESULTS BY MEANS OF A SAMPLE PLAN/WINDOW
FOR AEROBIC COLONY COUNTS WITH RESET WHERE $n=35$, $c=7$, $m=3162$
 cfu/cm^2 ($\log 3.5$), $M=100,000 \text{ cfu/cm}^2$ ($\log 5$)**

MICROBIOLOGICAL SAMPLE RECORD FORM							
Establishment:		Microbiological results for:			Total	Viable	Page no:
Date	Species	Sample (product)/Laboratory reference	type	Sample number/window number	Result (cfu/g)	Indicate: Acceptable/Marginal/Fail	Number of marginal results (Between m and M)
2009.08.01	Ostrich	Carcass		1 (1st window)	501	Acceptable	
2009.08.01	Ostrich	Carcass		2	662	Acceptable	
2009.08.01	Ostrich	Carcass		3	1004	Acceptable	
2009.08.01	Ostrich	Carcass		4	3161	Acceptable	
2009.08.01	Ostrich	Carcass		5	3009	Acceptable	
2009.08.01	Ostrich	Primal cuts		6	2990	Acceptable	
2009.08.01	Ostrich	Retail portions		7	765	Acceptable	
2009.08.08	Ostrich	Carcass		8	328	Acceptable	
2009.08.08	Ostrich	Carcass		9	3200	Acceptable	
2009.08.08	Ostrich	Carcass		10	3162	Acceptable	
2009.08.08	Ostrich	Carcass		11	3170	Marginal	1
2009.08.08	Ostrich	Carcass		12	3456	Marginal	2
2009.08.08	Ostrich	Primal cuts		13	31	Acceptable	
2009.08.08	Ostrich	Retail portions		14	567	Acceptable	
2009.08.15	Ostrich	Carcass		15	8097	Marginal	3
2009.08.15	Ostrich	Carcass		16	97896	Marginal	4
2009.08.15	Ostrich	Carcass		17	8767	Marginal	5
2009.08.15	Ostrich	Carcass		18	7865	Marginal	6
2009.08.15	Ostrich	Carcass		19	3134	Acceptable	
2009.08.15	Ostrich	Primal cuts		20	2800	Marginal	7
2009.08.15	Ostrich	Retail portions		21	3567	Marginal	8 ⁽²⁾
2009.09.22	Ostrich	Carcass		1 (2 nd Window)	350	Acceptable	
2009.09.22	Ostrich	Carcass		2	356	Acceptable	
2009.09.22	Ostrich	Carcass		3	567	Acceptable	
2009.09.22	Ostrich	Carcass		4	234	Acceptable	
2009.09.22	Ostrich	Carcass		5	123	Acceptable	
2009.09.22	Ostrich	Primal cuts		6	657	Acceptable	
2009.09.22	Ostrich	Retail portions		7	10345	Failed	Failed ⁽³⁾
2009.09.29	Ostrich	Carcass		1 (3 rd Window)	978	Acceptable	
2009.09.29	Ostrich	Carcass		2	879	Acceptable	
2009.09.29	Ostrich	Carcass		3	767	Acceptable	
2009.09.29	Ostrich	Carcass		4	645	Acceptable	
2009.09.29	Ostrich	Carcass		5	645	Acceptable	
2009.09.29	Ostrich	Primal cuts		6	667	Acceptable	
2009.09.29	Ostrich	Retail portions		7	367	Acceptable	⁽⁴⁾

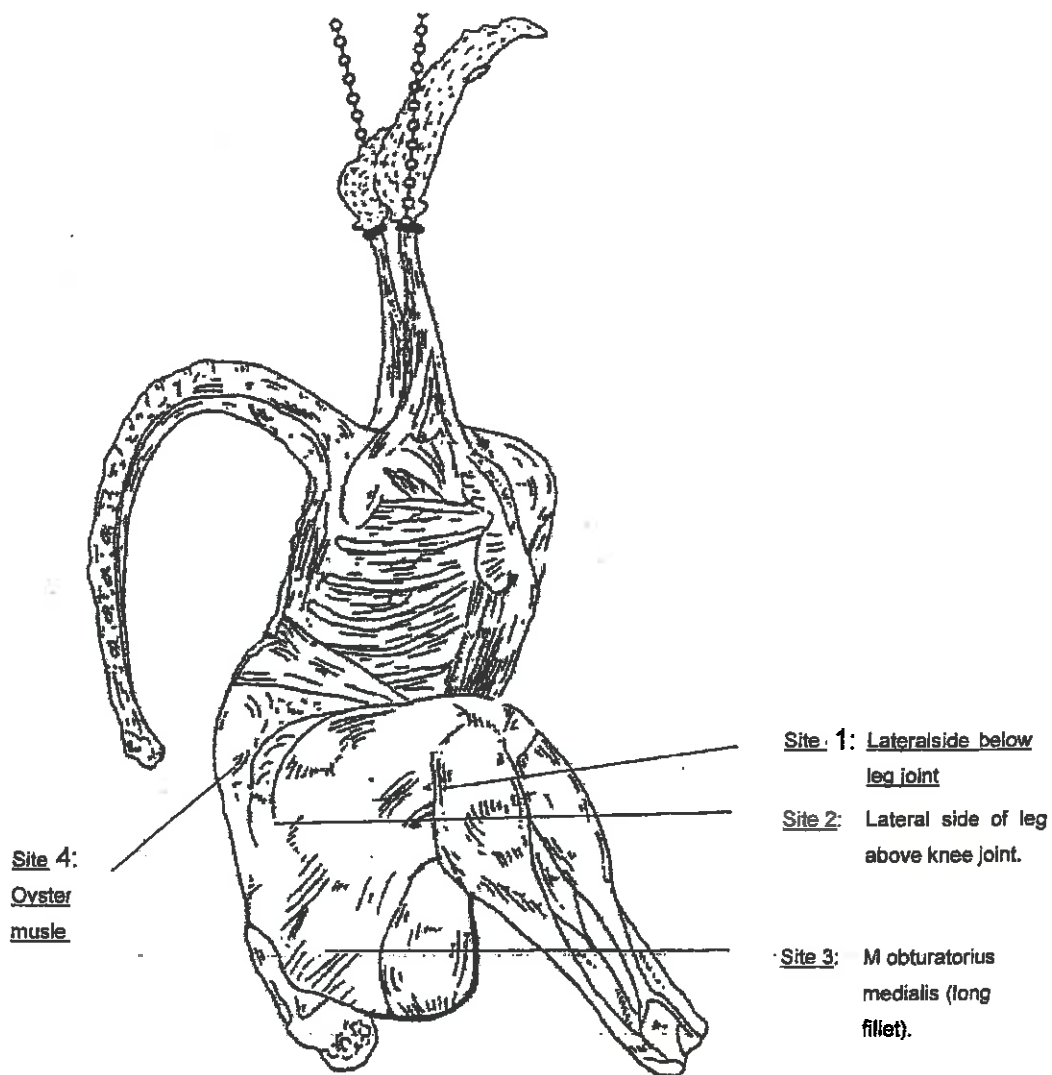
- (1) Delete as appropriate (2) Window 1 failed because c exceeded 7. A new window is started. (3) Window 2 failed because sample no. 7 exceeded M . A new window is started. (4) Please note that n is always measured from the last 35 sample results listed. (The window used for evaluation is dragged down the list every time new results are added to the data.)

Annex 5: EXPLANATION OF EVALUATION OF MICROBIOLOGICAL RESULTS BY MEANS OF A SAMPLE PLAN/WINDOW FOR AEROBIC COLONY COUNTS WITH RESET WHERE $n=35$, $c=7$, $m=3162 \text{ cfu/cm}^2 (\log 3.5)$, $M=100,000 \text{ cfu/cm}^2 (\log 5)$

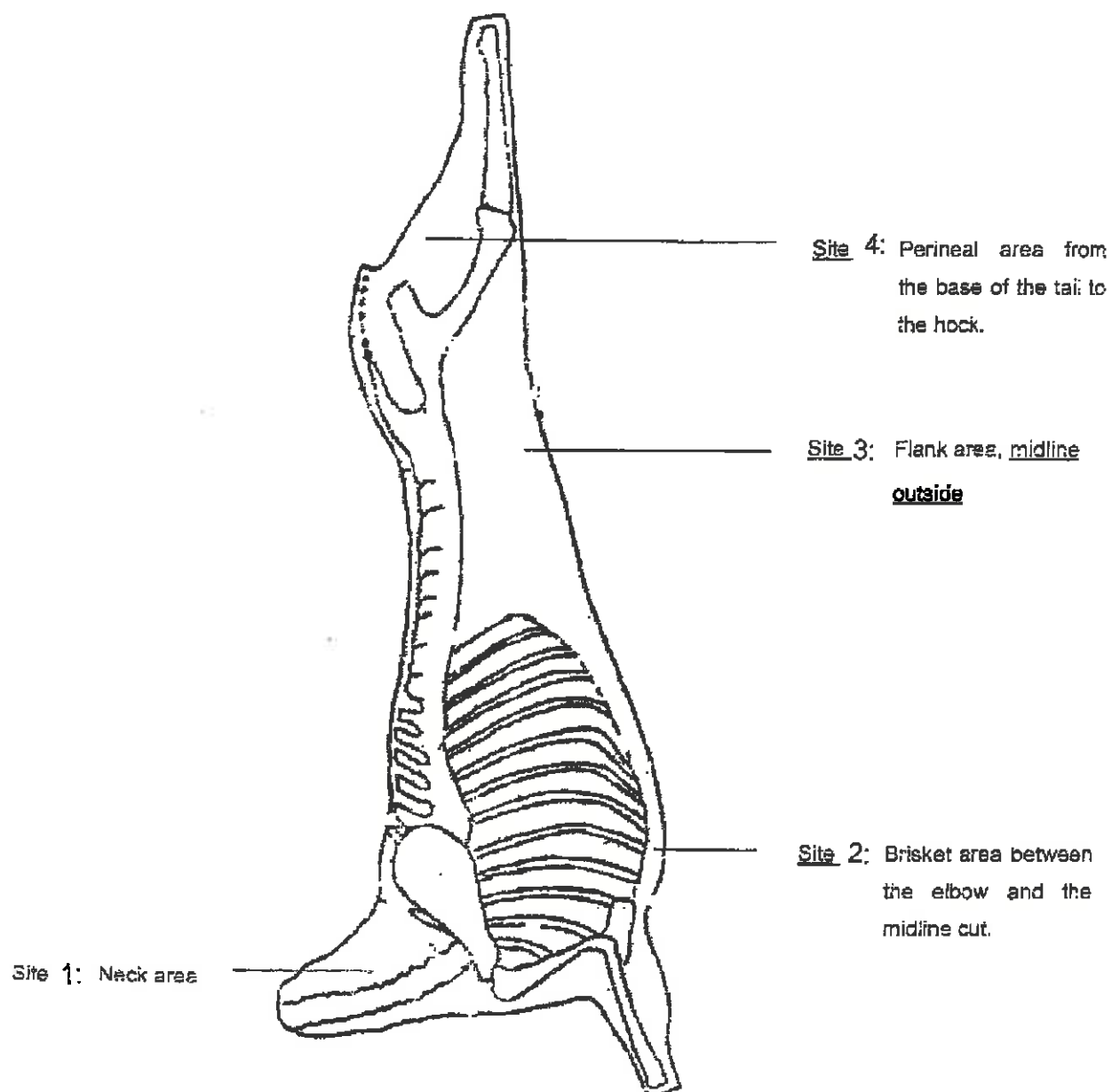
Plant No.	Sample No.	ACC	Moving Window	Sample No.	ACC	Moving Window	Sample No. Results?	Moving Window	
ZA 13	1	300	1 st Window						
ZA 13	2	2500							
ZA 13	3	800							
ZA 13	4	800							
ZA 13	5	600							
ZA 13	6	600							
ZA 13	7	10 000							
ZA 13	8	300							
ZA 13	9	200							
ZA 13	10	6000							
ZA 13	11	7000							
ZA 13	12	900							
ZA 13	13	11000							
ZA 13	14	200							
ZA 13	15	4000							
ZA 13	16	500							
ZA 13	17	12000	Failure(results >m<M but 'c' exceeded)						
ZA 13	18			1	300	2 nd Window			
ZA 13	19			2	800				
ZA 13	20			3	3000				
ZA 13	21			4	200				
ZA 13	22			5	350				
ZA 13	23			6	600				
ZA 13	24			7	400				
ZA 13	25			8	150000		Failure(result >M)		
ZA 13	26			9			1	And so on	
ZA 13	27			10			2	3 rd Window	
ZA 13	25			11			3		
ZA 13	29			12			4		
ZA 13	30			13			5		
ZA 13	31			14			6		
ZA 13	32			15			7		
ZA 13	33			16			8		
ZA 13	34			17			9		
ZA 13	(n) 35			18			10		
ZA 13				19			11		
ZA 13				20			12		
ZA 13				21			13		
ZA 13				22			14		
ZA 13				23			15		
ZA 13				24			16		
ZA 13				25			17		
ZA 13				26			18		
ZA 13				27			19		
ZA 13				28			20		
ZA 13				29 (up to 35)			21 (up to 35)		

1st Window failed because c exceeded 7 at sample No. 17. A new window is started. 2nd Window failed because sample no. 8 exceeded M . A new window is started, which is 3rd Window, and so on. Please note that n is always measured from the last 35 sample results listed. (The window used for evaluation is dragged down the list every time new results are added to the data.)

Annex 6: Bacteriological sampling sites on ostrich carcasses



Annex 7: Bacteriological sampling sites on sheep, calf, goat and small wild game carcasses



Annex 8: Figure 1. Bacteriological sampling sites on cattle, soliped and large wild game carcasses. Figure 2 Bacteriological sampling sites on pig carcasses

