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Microbiology of the food chain — Carcass sampling for microbiological analysis

Microbiologie de la chaîne alimentaire — Prélèvement d'échantillons sur des carcasses en vue de leur analyse microbiologique

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 17604:2003), which has been technically revised. It also incorporates the Amendment ISO 17604:2003/Amd.1:2009.

Introduction

It is generally agreed that the determination of microbial counts and the prevalence and/or numbers of pathogenic microorganisms on carcasses is essential for validation and verification in risk-based slaughter hygiene assurance systems [e.g. those employing the hazard analysis critical control points (HACCP) principles and quality assurance systems].

Moreover, many laboratories are involved in (regional, national, and international) monitoring or surveillance programmes on the prevalence and/or counts of pathogenic microorganisms to gather information for risk assessment. The design of such monitoring and surveillance programmes will benefit from the use of standardized and internationally accepted sampling procedures.

A harmonized sampling method, as described in this International Standard, can also be of interest for trade in meat and meat products.

Microbiology of the food chain — Carcass sampling for microbiological analysis

1 Scope

This International Standard specifies sampling methods for the detection and enumeration of microorganisms on the surface of carcasses or parts of carcasses of slaughtered meat animals. The microbiological sampling can be carried out as part of

— process hygiene control (to validate and or verify process control, e.g. total counts and *Enterobacteriaceae*) in slaughter establishments for large mammals, poultry and game,

— risk-based assurance systems for product safety, and

— monitoring or surveillance programmes for the prevalence and/or numbers of pathogenic microorganisms.

This International Standard includes the use of excision and swabbing techniques depending on the reason for sample collection. It also includes the use of carcass rinsing for the examination of carcasses of poultry and some small animals. Annex A shows sampling sites on the carcasses of various animal species.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
carcass
body of an animal after slaughter and dressing

3.2
excision technique
removal of measured areas of the surface tissue or skin by cutting

3.3
game
wild mammal or bird that is hunted for human consumption and farmed mammal or bird, including ratite (e.g. ostrich, emu), other than domestic ungulates and birds

3.4
large mammal
cattle (including buffaloes, bison), sheep, pigs (including wild boar), various types of deer (including reindeer, antelopes), horses (including donkeys and mules)

3.5
poultry
small-sized or medium-sized bird

EXAMPLE chicken, duck, goose, turkey, pigeon, pheasant, quail, grouse.

3.6
sampling plan
description of the examination of a required number of sample units by a defined analytical method, including the criteria for acceptance

3.7
sampling point
stage on the production line where a sample is taken

3.8
sampling site
place on the carcass where a sample is taken

3.9
small mammal and other small animal
lagomorph (e.g. hare, rabbit), rodent, turtle, frog

3.10
swabbing method
technique using absorbent material attached to the end of a stick or wire, or a sponge or piece of absorbent material, to pick up microorganisms from surfaces

4 General principles

The choice of sampling method depends mainly on the aim of the microbiological examination, the sensitivity required, and practical considerations. Excision, swabbing, and rinsing methods may be used, as permitted by this International Standard.

The excision of surface tissue generally harvests a higher number of microorganisms from the surface than other methods. Not all the microorganisms harvested will grow on the media and under the incubation conditions used. The repeatability and reproducibility of excision and rinsing methods are less variable than swabbing, because swabbing methods are more difficult to standardize.

However, only a small proportion of the carcass is sampled by excision methods, which might result in significant inaccuracies when total contamination is low and heterogeneously distributed, or when the presence of the target microorganisms is sparse. Excision methods are destructive, and can sometimes affect the value of the meat, but are preferred when sampling frozen surfaces.

Swabbing or rinsing techniques enable the examination of larger areas. Smaller areas targeting verified areas of greatest contamination may be examined using either excision or swabbing methods. Rinsing the whole carcass is an effective and practicable method for the examination of poultry (excluding large bird carcasses) and carcasses of some small mammals and other small animals.

5 Sampling plans

Sampling plans should relate to the purpose of testing and be applied according to the circumstances. They are not considered in detail.

The process stage, the time since start-up of the slaughtering process, the frequency of sampling, and, if relevant, the following, should be taken into account:

— the slaughterhouse practices for each animal;

— the design of risk-based process control assurance or harmonized monitoring programmes;

— the production volume;

— previous results of monitoring (trend analysis);

— the prevalence of relevant pathogenic microorganisms in the region from which the animal originates;

— relevant national, regional and/or international regulations.

In the case of process control, the time and frequency of sampling should relate to the level of slaughter hygiene.

In the case of monitoring and surveillance for pathogens, the sampling time, sites on the carcass, and frequency should maximize the chance of detecting and/or counting the pathogens sought.

6 Sampling points on the production line

Sampling points should be selected according to risk-based principles, and relate, when relevant, to the higher probability of detecting contamination during the process or at points in the slaughter process, as appropriate to measure the hygiene of specific production steps or the entire slaughter process. Examples of sampling points are the following:

— after the carcass polishing machine (pigs);

— after the carcass washing machine (pigs and poultry);

— after flaying (dehiding) (large mammals, game slaughtered in an abattoir, and others);

— after evisceration (all animals);

— immediately before chilling or freezing (all animals);

— immediately after chilling (poultry, small mammals, and other small animals);

— after chilling or freezing (all animals);

— in the chill room (all animals).

During the chilling period, depending on the chill room conditions, microorganisms might become sublethally damaged or die, might be overgrown by psychrotrophic microorganisms, or they might become more firmly attached to the meat, resulting in underestimation. This effect will be reduced if the sampling is carried out as soon as possible after slaughter.

7 Sampling sites on carcasses

7.1 Large mammals

The sampling sites chosen depend on the slaughterhouse practices, which can vary for different slaughterhouses and for different animals. The purpose is to examine the sites with the highest prevalence and/or level of contamination (see Table A.1). Figure A.1, Figure A.2, and Figure A.3 illustrate the sites most often identified as more highly contaminated. Other sampling sites may be specified in regulations, in guides of good practices, or in other technical guides for the sector. Consistency in the sampling sites over time is important to detect changes in the pattern of observations over a period of time (trend analysis). It is usually preferable to sample more carcasses at the sites most likely to

be contaminated, rather than more sites per carcass. Prevalence determinations in surveillance programmes will generally benefit from larger sampling areas.

7.2 Poultry, small mammals, and other small animals

A common method is to rinse the whole of these small carcasses. If surface samples are taken, the sites chosen depend on the slaughtering practice and equipment used. For poultry, neck skin or, if not present, breast skin is usually sampled.

7.3 Game

For larger game, the sampling sites can be similar to those for large mammals (7.1). In general, for each species samples are to be collected from sites most likely to be contaminated.

8 Sampling techniques

8.1 General

For a given sampling situation, the same sampling technique shall be used each time, to ensure that results are comparable. In general, three different methods can be used, the excision (destructive) method, the swabbing (non-destructive) method or the rinsing method. As the surface of the carcass is being sampled, the results are expressed in terms of colony forming units (cfu) per cm^2. When using the rinsing technique, the results are usually expressed as cfu per carcass. When sampling skin from poultry carcasses, the results are expressed as cfu/g.

A number of samples from one carcass, or from several carcasses at the same sampling site, can be combined to make one pooled sample that is taken as a whole for analysis in the laboratory. Alternatively, a number of samples from one carcass can be combined to one composite sample, from which a test portion is taken for analysis (see ISO 6887-1).

8.2 Excision methods

In general, two different methods are used, the cork borer and the template method. Both sample the surface of the meat (as the inner part of the meat is normally sterile). The cork borer usually samples smaller areas than the template method, but is easier to use when examining frozen meat.

8.2.1 Cork borer method

8.2.1.1 Reagent

8.2.1.1.1 Ethanol, 70 % volume fraction.

8.2.1.2 Equipment

8.2.1.2.1 Sterile scalpels.

8.2.1.2.2 Sterile forceps.

8.2.1.2.3 Sterile cork borers, with a cutting area of at least 5 cm^2 (diameter about 2,5 cm).

8.2.1.2.4 Sterile scissors.

8.2.1.2.5 Portable gas blow torch or **portable Bunsen burner** (optional).

8.2.1.2.6 Tissues or **cotton wool**.

8.2.1.2.7 Sterile plastic bags, for a peristaltic type or sonic homogenizer of appropriate size for the area being sampled and the volume of diluent to be added.

8.2.1.3 Collection of samples

At the relevant sites on the carcass, circular incisions are made in the surface with a sterile cork borer (8.2.1.2.3). The cork borer is removed and discs of skin or tissue (approximately 2 mm thick) are then cut loose from the surface end with a sterile scalpel (8.2.1.2.1) or scissors (8.2.1.2.4) and forceps (8.2.1.2.2) and put into a labelled sterile plastic bag (8.2.1.2.7).

8.2.1.4 Cleaning and sterilization of equipment

Each sample shall be taken using clean and sterile equipment (8.2.1.2). Sterilization can be achieved, e.g. by autoclaving suitably wrapped equipment. Equipment may be reused during sampling, provided it is carefully cleaned and disinfected between sampling. This may be done as follows:

a) clean with tissues or cotton wool (8.2.1.2.6) dipped in 70 % ethanol (8.2.1.1.1);

b) dip in 70 % ethanol in a bottle;

c) burn the ethanol off (8.2.1.2.5)(if the use of a naked flame is hazardous, then, merely allow the ethanol to evaporate);

d) allow to cool.

Due to the time needed to carry out the cleaning, it is useful to have several sets of pre-sterilized equipment (e.g. cork borer, scalpels, and forceps) available. It is essential that these tools are not re-contaminated before use. As an alternative, use sterile disposable instruments.

NOTE If samples are combined into a pooled or composite sample, it is not necessary to clean and disinfect between taking these samples.

8.2.2 Template excision method

This method is identical to the cork borer method (8.2.1), except for the use of templates and scalpel or knife instead of a cork borer. Templates are usually made from metal or plastic, and are used to define the sampling area, e.g. 10 cm^2, 50 cm^2, or 100 cm^2.

8.2.3 Skin sampling

8.2.3.1 Neck skin

Neck skins are often removed from poultry carcasses as they pass on the production line, so they have to be cut off rapidly, but may be trimmed later and weighed (individually or as a composite sample). Take a pair of sterile scissors (8.2.1.2.4). Open a sterile plastic bag (8.2.1.2.7) without touching the sterile interior of the bag. Grip the bag at the bottom seam and fold it back over the hand so that it is inside out. Avoiding carcasses with very short neck skins, grip the neck skin of a carcass firmly through the bag and cut it off as rapidly as possible. This is usually done using scissors. Measure the sample size by weighing (one neck skin weighs about 10 g). If necessary, several samples shall be combined to give the desired sample size, e.g. 25 g or 50 g.

8.2.3.2 Breast skin

It is quick and easy to remove the skin from poultry breasts. Take the carcass to be sampled and put it on a flat surface, avoiding any contact with the parts of the skin to be sampled. Remove as much of the breast skin as possible using scalpel (8.2.1.2.1) and forceps (8.2.1.2.2) and weigh it. The results are then expressed with respect to the mass (as cfu per gram or as presence/absence in 25 g).

8.3 Swabbing methods

Swabbing is a non-destructive method especially used for sampling larger areas. The technique includes the use of sticks with absorbent material, swabs, tampons, sponges, and cloths, mainly depending on the circumstances and area to be examined.

8.3.1 Wet and dry stick swab method

8.3.1.1 Reagent

8.3.1.1.1 Sterile diluent, for general use (see ISO 6887-1), dispensed in 10,0 ml amounts in tubes or bottles.

8.3.1.2 Equipment

8.3.1.2.1 Sterile cotton wool swabs with wooden shafts.

The size depends on the area to be swabbed (e.g. small swabs for 10 cm^2 on small game carcasses, large swabs for 50 cm^2 on beef carcasses).

8.3.1.2.2 Sterile square templates (see 8.2.2).

8.3.1.3 Collection of samples

Moisten a swab (8.3.1.2.1) in 10 ml diluent (8.3.1.1.1). At a selected carcass-sampling site, press the template (8.3.1.2.2) hard on to the surface. Rub the swab over the whole area using pressure, moving in at least two directions, e.g. firstly horizontally and then vertically, at least 10 times in each direction. Place the swab into the diluent used to wet the swab, breaking off the wooden shaft against the inside of the bottle. Then, with a dry swab, sample the same area again, using the same technique to absorb any liquid remaining from the wet swab as above, and place this swab into the same container of diluent.

The templates may be cleaned, disinfected, and reused as described under 8.2.1.4.

8.3.2 Sponge, tampon swab, and small cloth method

Sponges and tampon swabs are used particularly for the sampling of larger areas. Sampling of larger non-template areas can increase the chance of detecting pathogens that are present at low levels on the carcasses. More than one site may be sampled using the same swab.

8.3.2.1 Reagent

8.3.2.1.1 Sterile diluent, for general use (see ISO 6887-1).

8.3.2.2 Equipment

8.3.2.2.1 Sterile specimen sponge (free of inhibitory substances), 25 cm^3 to 50 cm^3 in a sterile plastic bag. Large cellulose sponges may also be suitable.

8.3.2.2.2 Sterile tampon swabs.

These swabs typically comprise large composite fabric (cloth) swabs with multiple layers of gauze or cotton wool encased in gauze. Sanitary towels or tampons (free of inhibitory substances), which are constructed in this way, are often used.

8.3.2.2.3 Sterile small cloth (free of inhibitory substances).

8.3.2.2.4 Plastic bags, for a peristaltic type or sonic type homogenizer.

8.3.2.2.5 Sterile template, with hollow internal area of not less than 100 cm^2.

8.3.2.2.6 Sterile gloves.

8.3.2.3 Collection of samples.

At the sampling point, open the plastic bag (8.3.2.2.4) containing the sterile sponge (8.3.2.2.1), sterile tampon swab (8.3.2.2.2), or sterile small cloth (8.3.2.2.3) and add sufficient diluent (8.3.2.1.1) from a defined volume of diluent (e.g. 25 ml or 100 ml) to wet the sponge or the tampon swab without excess fluid being evident. Massage the sponge or the tampon swab from outside the bag to moisten it thoroughly. Place the template (8.3.2.2.5) over the test area. Either use the bag as a glove by turning it inside out and grasping the sponge or tampon through the bag, as for taking neck skins (8.2.3.1), or use a fresh pair of sterile gloves (8.3.2.2.6), to wipe the sponge or tampon swab over the test surface. Sample by rubbing in at least two directions, e.g. firstly horizontally and then vertically, at least 10 times in each direction. After swabbing, place the sponge or the tampon swab back in its plastic bag and add the remainder of the diluent.

The template may be reused as described under 8.2.1.4.

8.4 Carcass rinsing method

8.4.1 Reagent

8.4.1.1 Sterile diluent, e.g. in 300 ml, 400 ml, or 600 ml volumes.

8.4.2 Equipment

8.4.2.1 Large stomacher type plastic bags.

8.4.3 Collection of samples

8.4.3.1 Poultry

Carcasses are normally taken off the moving production line. Open a large stomacher type bag (8.4.2.1) without touching the sterile interior. Enclose a carcass with the bag while it is still on the line, and, using both hands while holding the legs of the carcass through the bag, lift the carcass off the line (i.e. detach its legs from the shackles). Try to avoid taking carcasses with significant volumes of water still draining off them. If such carcasses are taken, remove them, under aseptic conditions, to a separate, disinfected set of shackles and allow the water to drain off before enclosing it in a bag.

Rest the bottom of the bag containing the carcass on a flat surface. Holding the top of the bag slightly open, add sterile diluent (8.4.1.1), usually 400 ml (chickens) or 600 ml (turkeys), to the bag, pouring the solution into the carcass cavity and over the exterior of the carcass. Expel most of the air from the bag and then close the top of the bag with, e.g. a tie wrap. Holding the bag securely, rinse the carcass inside and out, using a rocking motion, for approximately 1 min. Do this by holding the carcass through the bottom of the bag with one hand and the closed top of the bag with the other hand. Holding the carcass securely in this way, move it through an arc, shifting the weight of the carcass from one hand to the other to ensure that all surfaces (interior and exterior) of the carcass are rinsed. Rest the bag with the carcass on a flat surface and, while supporting the carcass, open the bag. Remove the carcass from the bag, firstly letting any excess fluid drain back into the bag. Take care not to touch the interior of the bag. Secure the top of the bag so that the rinse fluid will not spill out or become contaminated. Alternatively, transfer the rinse fluid under aseptic conditions from the bag to a sterile container (e.g. the bottle that contained the sterile diluent). This may be done without taking the carcass from the bag.

9 Storage and transport of samples

Transport the samples in an insulated cool box with frozen freezer blocks, a crushed melting ice cool box, or a (portable) refrigerator. Do not allow the samples to freeze or to be exposed to the frozen blocks, if used. Keep the temperature between 1 °C to 8 °C.

Examine the samples as soon as possible after receipt, or store them at 3 °C ± 2 °C for a maximum of 24 h (see ISO 7218).

10 Microbiological examination of samples

10.1 General

For general rules for microbiological examinations, see ISO 7218.

10.2 Initial preparation of samples

When sampling using one of the various types of swab, the microbes shall be released from the swab by adding an appropriate volume of liquid (diluent, broth, or enrichment medium) and agitating and/or massaging through the plastic sample bag.

10.2.1 Swabbing methods

For general preparation and calculation instructions for swabs, refer to ISO 18593.

10.2.1.1 Wet swab/dry swab technique

The contents of the two swabs are extracted into the diluent by agitating on a vortex type mixer before dilution and plating in accordance with ISO 6887-1. Sterile glass beads may be added to aid this process.

10.2.1.2 Sponge, tampon swab, and small cloth method

The bag containing the sponge, tampon swab, or small cloth and the diluent is placed in a peristaltic homogeniser for 1 min, or on a homogenizer using horizontal shaking for 30 s, before dilution and plating in accordance with ISO 6887-1.

10.2.2 Excised samples

The preparation of excised samples shall be performed in accordance with ISO 6887-2. For the determination of low numbers, it is useful to prepare the initial suspension in lower ratios [e.g. 1:1 (1 in 2) or 1:4 (1 in 5)] instead of the routine 1:9 (1 in 10).

10.2.3 Carcass rinse samples

Mix thoroughly by shaking before diluting and plating in accordance with ISO 6887-1.

10.3 Enumeration and detection of microorganisms

These shall be performed in accordance with the relevant standard methods. The quantitative results from samples obtained by excision or swabbing shall be expressed as colony forming units (cfu) per square centimetre and not cfu per gram or per millilitre, with the exception of results from poultry skin, which can be expressed as cfu per gram. For carcass rinse samples, the results of plate counts shall be expressed as cfu per ml carcass rinse stating the volume of the rinse liquid, or as cfu per carcass. In the case of enrichment procedures, report the target microorganism as detected or not detected in the area/test portion analysed or per carcass tested.

Annex A
(informative)

Sampling sites

The sampling sites to be chosen depend on the slaughterhouse practices for different animals. The purpose is to examine the sites with the highest prevalence of contamination (see Table A.1). Figure A.1, Figure A.2, and Figure A.3 show examples of the sampling sites on the surface of the carcass of pigs, cattle, and sheep respectively. For other large animals, e.g. horses, deer, and similar animals, sampling sites will be similar to those for cattle. However, this should be verified. The slaughter techniques used will affect the sites most highly contaminated.

Table A.1 — Sites most often identified as more highly contaminated

Pigs[a]	Cattle[a]	Sheep[a]
1 Pelvic channel internal	1 Pelvic channel internal	1 Knee external aspect
2 Pelvic channel external	2 Pelvic channel external	2 Pelvic channel internal
3 Abdominal	3 Hock external aspect	3 Abdominal external
4 Xiphoid external	4 Hock internal aspect	4 Anterior sternum external
5 Xiphoid internal	5 Internal thigh	5 Foreleg, elbow, external aspect
6 Pillar of diaphragm	6 Sternum external	6 Neck, prescapular region external
7 Submaxillary external	7 Sternum internal	
8 Submaxillary internal	8 Xiphoid external	
9 Fore foot external aspect	9 Xiphoid internal	
10 Fore foot internal aspect	10 Foreleg internal aspect	
	11 Atlanto-occipital internal aspect	
	12 Atlanto-occipital external aspect	

a The numbers 1 to 12 indicate the sampling sites in Figure A.1 to Figure A.3.

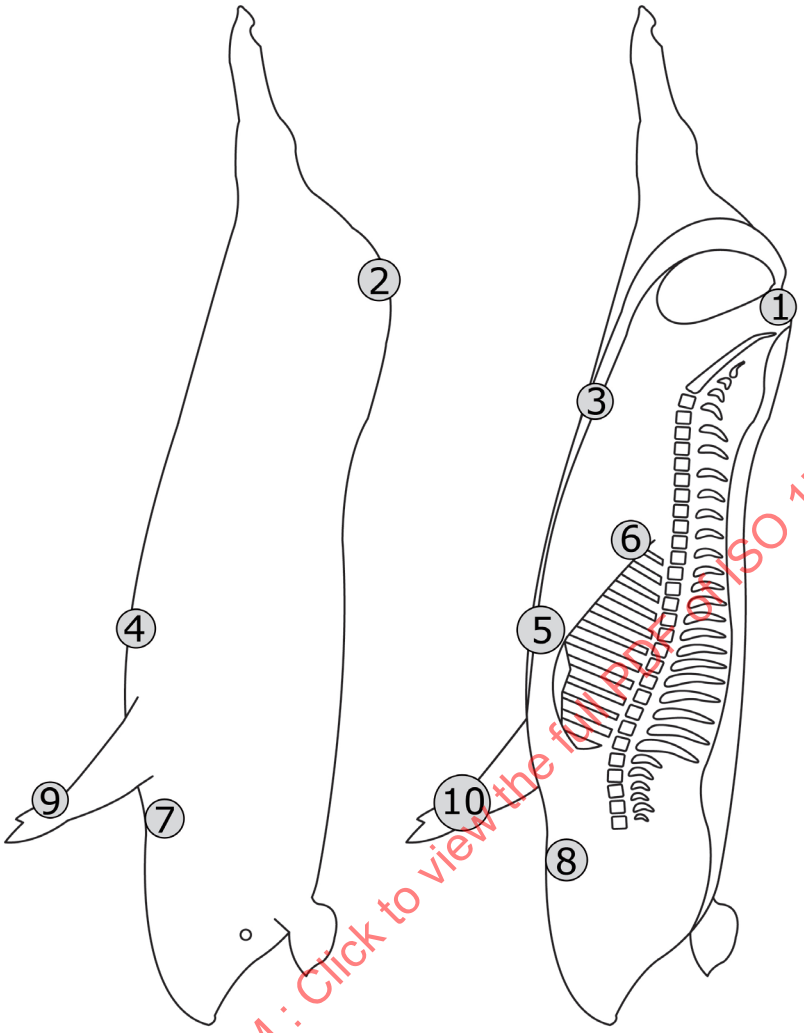

Figure A.1 — Pig: examples of sampling sites (left = lateral, right = medial)