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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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Contents

Page

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).