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Microbiology of food and animal feeding stuffs — Carcass sampling for microbiological analysis

Microbiologie des aliments — Prélèvement d'échantillons sur des carcasses en vue de leur analyse microbiologique

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 17604 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 17604 cancels and replaces the second edition of ISO 3100-1:1991.

ISO 3100-2:1988 is under revision as ISO 6887-2.

Introduction

It is generally agreed that the determination of microbial counts and the prevalence of pathogenic microorganisms on carcasses is essential for monitoring 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 institutes are involved in (international) surveillance programmes on the prevalence of pathogenic microorganisms.

The design of such monitoring and surveillance programmes will obviously benefit from the use of standardized and internationally accepted sampling procedures.

Microbiology of food and animal feeding stuffs — Carcass sampling for microbiological analysis

1 Scope

This International Standard specifies sampling methods for the detection and enumeration of microorganisms on the carcass surface of freshly slaughtered (red) meat animals. The microbiological sampling can be carried out as part of

— the process control (and to verify process control) in slaughter establishments for cattle, horses, pigs, sheep, goats and game raised in captivity,

— risk-based assurance systems for product safety, and

— surveillance programmes for the prevalence of pathogenic microorganisms.

This International Standard includes the use of destructive and non-destructive techniques depending on the reason for the sample collection.

It does not consider the use of sampling plans.

When national legislation on the topic exists, this prevails over this International Standard.

Annex A shows sampling sites on the carcass, and Annex B gives requirements for microbiological examination. Annex C compares destructive and non-destructive methods.

2 Normative references

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

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 °C*

ISO 5552, *Meat and meat products — Detection and enumeration of* Enterobacteriaceae *without resuscitation — MPN technique and colony-count technique*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of* Salmonella *spp.*

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 rules for microbiological examinations*.

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive* Escherichia *coli — Most probable number technique*

ISO 10272, *Microbiology of food and animal feeding stuffs — Horizontal method for detection of thermotolerant* Campylobacter

ISO 10273, *Microbiology of food and animal feedings stuffs — Horizontal method for the detection of presumptive pathogenic* Yersinia enterocolitica

ISO 13720, *Meat and meat products — Enumeration of* Pseudomonas *spp.*

ISO 16654, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of* Escherichia coli *O157*

3 Sampling procedure

Both destructive and non-destructive methods may be used (see Annex C). Avoidance of adverse effects on the carcass value is the primary constraint on the use of destructive methods. Non-destructive techniques enable the examination of larger areas. Smaller areas targeted to proven areas of greatest contamination may be examined using either destructive or non-destructive methods (see C.2 and C.3).

4 Sampling frequency

The time and frequency of sampling is governed by

— the slaughterhouse practices for each animal,

— the design of risk-based process control assurance programmes,

— the production volume, and

— the epidemiological status of the region from where the animal originates.

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

In the case of surveillance for pathogens, the sampling time, location on the carcass, and frequency should correspond to the greatest chance of isolating the pathogens sought.

5 Sampling points

5.1 Carcass selection

Every carcass should have an equal chance of being selected for sampling.

5.2 Process control

Sampling points in the slaughterhouse should relate to the slaughter practices used. They should be selected according to risk-based principles, and relate to identified problem areas in the process. Examples of control points are the following:

— after the carcass polishing machine (pig);

— after the carcass washing machine (pig);

— after flaying (dehiding) (cattle, sheep, goat, game raised in captivity and others);

— after evisceration;

— in the chill room at least 12 h after slaughter (see C.4).

5.3 Detection of pathogenic microorganisms

For the detection of pathogenic microorganisms, the following sampling points may be used for all species:

— immediately before chilling;

— in the chill room at least 12 h after slaughter (see C.4).

6 Sampling sites

6.1 Process control

The sampling sites chosen depend on the slaughterhouse practices for different animals (see Figures A.1, A.2 and A.3). These sampling sites are not compulsory.

Consistency in the choice of sampling sites over time is important.

It is usually preferable to sample as many carcasses as possible at the expense of the number of sampling sites on the individual carcass.

6.2 Detection of pathogenic microorganisms

The sampling sites 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). These sampling sites are not compulsory.

Consistency in the choice of sampling sites over time is important.

It is usually preferable to sample as many carcasses as possible at the expense of the number of sampling sites on the individual carcass.

While prevalence determinations in surveillance programmes will generally benefit from larger sampling areas, sampling of smaller areas targeted to areas of greatest contamination may achieve the same result.

7 Sampling techniques

7.1 General

For a given sampling situation, the same sampling technique should be used each time, to ensure that results are comparable.

7.2 Destructive methods

7.2.1 Corkborer method

7.2.1.1 Reagents

7.2.1.1.1 Ethanol, 70 % and 90 % by volume.

7.2.1.2 Apparatus and materials

7.2.1.2.1 Sterile scalpels.

7.2.1.2.2 Sterile forceps.

7.2.1.2.3 Sterile corkborers, with a cutting area of 5 cm^2.

7.2.1.2.4 Portable gas blow torch or **portable Bunsen burner**.

7.2.1.2.5 Tissues or **cotton wool**.

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

7.2.1.3 Collection of samples

At the relevant places on the carcass, holes are made in the surface with a sterile corkborer (7.2.1.2.3). The discs of skin or tissue (approximately 2 mm thick) are then cut loose with a sterile scalpel and forceps and put into a labelled sterile plastic bag (7.2.1.2.6).

7.2.1.4 Cleaning and sterilization of materials

The corkborer (7.2.1.2.3), scalpel and forceps shall be cleaned and sterilized after each sampling as follows.

a) Clean with tissues or cotton wool dipped in 70 % ethanol (7.2.1.1.1).

b) Dip in 70 % ethanol in a bottle.

c) Burn the ethanol off; if the use of a naked flame is hazardous, then allow the ethanol to evaporate.

d) Allow to cool.

Due to the amount of time needed to carry out the cleaning, it is best to use at least two sets of corkborer, scalpel and forceps. It is essential that these tools are not re-contaminated before use. As an alternative, the use of sterile disposable materials is allowed.

7.2.2 Template excision method

7.2.2.1 Apparatus and materials

7.2.2.1.1 Sterile scalpels.

7.2.2.1.2 Sterile forceps.

7.2.2.1.3 Sterile square templates, with hollow internal area of, for example, 10 cm^2, 20 cm^2 or 25 cm^2.

7.2.2.1.4 Sterile plastic bags, for a peristaltic type homogenizer.

7.2.2.2 Collection of samples

At the relevant places of the carcasses, about 2 mm thick samples are cut delineated by sterile templates, using sterile scalpels and forceps.

The instruments may be re-used as described under 7.2.1.4.

7.3 Non-destructive methods

7.3.1 **Wet and dry swab method** (see reference [1])

7.3.1.1 Reagents

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

7.3.1.2 Apparatus and materials

7.3.1.2.1 Sterile cotton wool swabs, large size with wooden shaft.

7.3.1.2.2 Sterile square templates, with hollow internal area of, for example, 50 cm^2 or larger.

7.3.1.3 Collection of samples

Moisten a swab in 10 ml peptone salt diluent (7.3.1.1.1). At each selected carcass test side, press the template (7.3.1.2.2) hard onto the surface. Rub the swab over the whole area using pressure, moving first horizontally and turning the swab so that all sides are used. 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 area again, as above and place this swab into the same container of diluent.

The instruments may be re-used as described under 7.2.1.4.

7.3.2 Sponge sampling method

7.3.2.1 Reagents

7.3.2.1.1 Sterile peptone salt diluent, for general use (see ISO 6887-1), dispensed in 25,0 ml amounts in bottles.

7.3.2.2 Apparatus and materials

7.3.2.2.1 Sterile specimen sponge (free of inhibitory substances), in a sterile plastic bag.

7.3.2.2.2 Sterile square template, with hollow internal area of 100 cm^2 (10 cm × 10 cm).

7.3.2.2.3 Sterile gloves.

7.3.2.3 Collection of samples

Locate the sampling sites. Open the bag containing the sterile sponge (7.3.2.2.1) and add sufficient peptone salt diluent (7.3.2.1.1) to wet the sponge without excess fluid being visible. Massage the sponge from outside the bag to moisten it thoroughly. Put on a pair of sterile gloves and carefully remove the sponge from the bag.

Place the template (7.3.2.2.2) over the location. Wipe the sponge over the enclosed sampling site (10 cm × 10 cm) for a total of approximately 10 times in the vertical and 10 times in the horizontal direction.

After swabbing, place the sponge back in the sponge sample bag. Add further diluent to the sample bag to make a total of 25 ml.

The instruments may be re-used as described under 7.2.1.4.

7.3.3 Gauze tampon method

7.3.3.1 Reagents

7.3.3.1.1 Sterile peptone salt diluent, for general use (see ISO 6887-1), dispensed in 25,0 ml amounts in bottles.

7.3.3.2 Apparatus and materials

7.3.3.2.1 Sterile gauze tampon.

7.3.3.2.2 Sterile plastic bags, for a peristaltic-type homogenizer.

7.3.3.2.3 Sterile square template, with hollow internal area of 100 cm^2 (10 cm × 10 cm).

7.3.3.2.4 Sterile gloves.

7.3.3.3 Collection of samples

At the sampling site, open the plastic bag containing the tampon (7.3.3.2.1) and add about 10 ml of peptone salt diluent (7.3.3.1.1). Squeeze and massage the tampon from outside the bag to thoroughly moisten it. Place the template (7.3.3.2.3) over the test area. Either hold the bag outside and turn inside out (use as a glove) or use a fresh pair of sterile gloves to wipe the tampon over the test surface, 10 times in the horizontal direction then 10 times in the vertical direction. Place the tampon back in its plastic bag and add further diluent to make a total of 25 ml.

The instruments can be re-used as described under 7.2.1.4.

8 Storage and transport of samples

Transport the samples in an insulated cool box with frozen freezer blocks or a crushed melting ice cool box. Do not allow the samples to freeze or to come into contact with the frozen blocks of ice, if used.

Either process the samples in the laboratory within 1 h of collection or store them at 2 °C ± 2 °C for a maximum of 24 h (see ISO 7218).

Annex A
(informative)

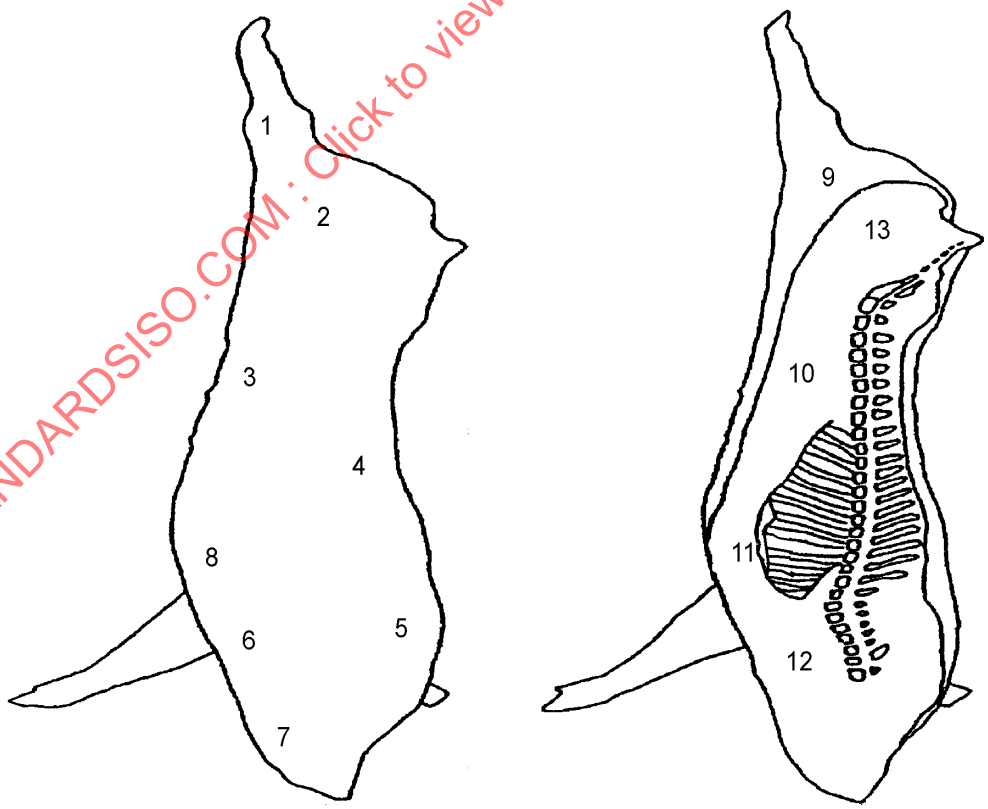
Sampling sites

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). Figures A.1, A.2 and A.3 show examples of the sampling sites on the surface of the carcass of pig, beef and lamb respectively (see reference [2]).

Table A.1 — Sites most consistently contaminated by high numbers of microorganisms

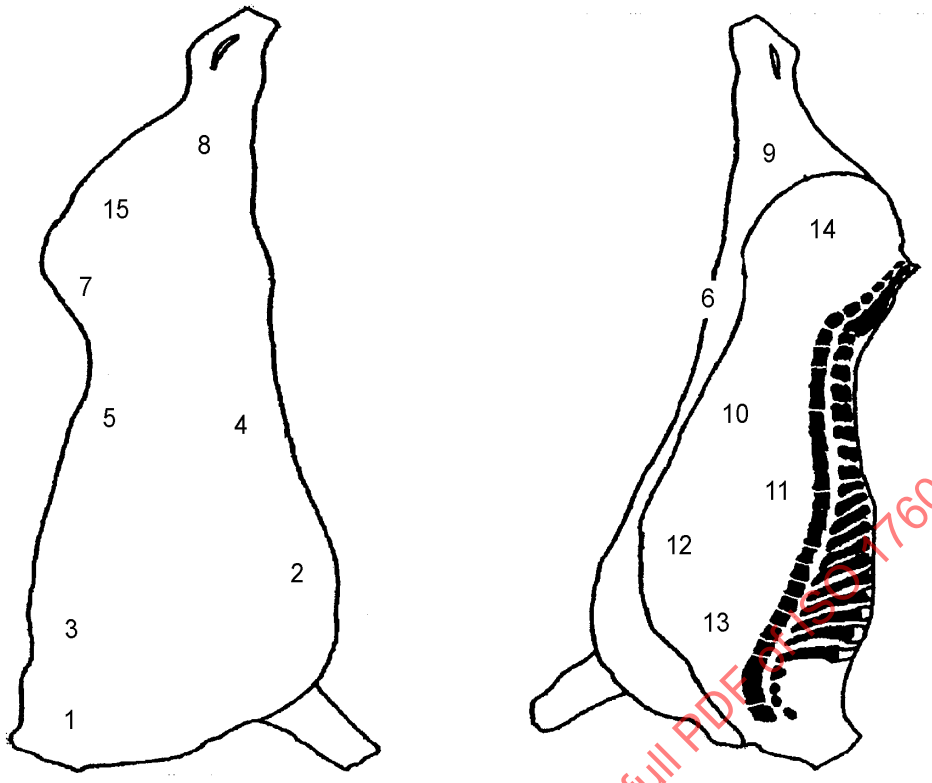
Pig [a]	Beef [a]	Lamb [a]
Distal hind limb (trotter) (1) [a]	Brisket (2)	Abdomen (flank) (3)
Hind limb, lateral (2)	Forerib (3)	Thorax, lateral (4)
Abdomen, lateral (belly) (3)	Flank (4)	Crutch (6)
Mid-dorsal region (mid-back) (4)	Flank groin (6)	Breast, lateral (7)
Abdomen, medial (10)	Round, lateral (8)	

[a] The numbers (x) indicate the sampling sites in Figures A.1 to A.3.



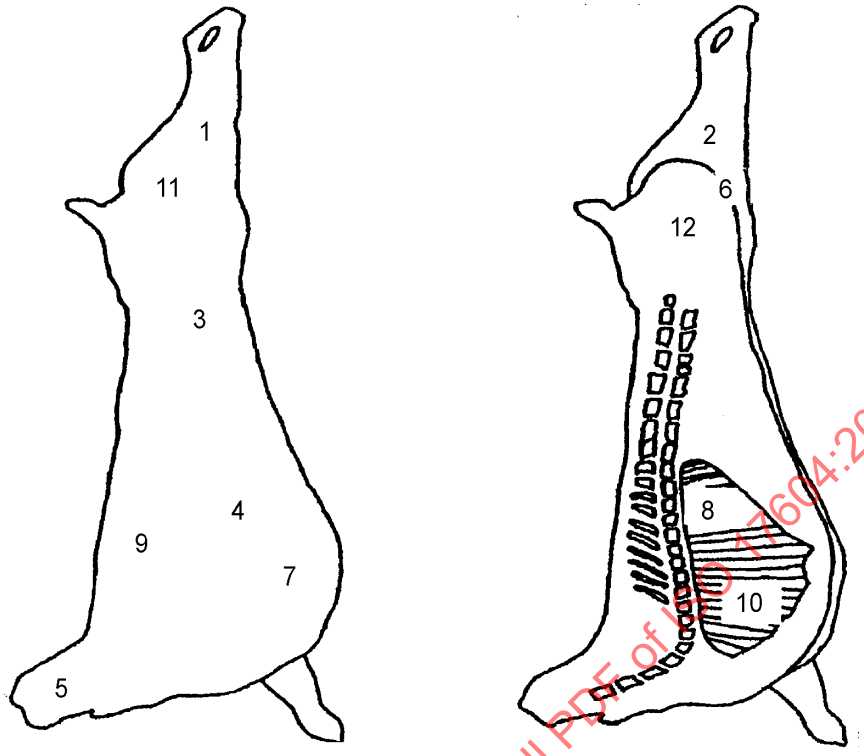
a) Lateral **b) Medial**

Figure A.1 — Pig: Examples of sampling sites



a) Lateral b) Medial

Figure A.2 — Beef: Examples of sampling sites



a) Lateral b) Medial

Figure A.3 — Lamb: Examples of sampling sites

Annex B
(normative)

Microbiological examination

B.1 Preparation of test samples

The preparation shall be performed in accordance with ISO 6887-2. For general rules for microbiological examinations, see ISO 7218.

B.2 Process control

Colony counts per square centimetre of carcass surface shall be performed in accordance with ISO 4833. Enumeration of *Enterobacteriaceae* shall be performed in accordance ISO 5552, presumptive *Escherichia coli* in accordance ISO 7251, and *Pseudomonas* in accordance with ISO 13720.

The above-mentioned methods for process control shall be adapted to colony counts per square centimetre instead of colony-forming units per gram or per millilitre.

B.3 Surveillance for pathogenic microorganisms

Pathogenic microorganisms may be determined qualitatively.

Salmonella shall be determined in accordance with ISO 6579, *Campylobacter* in accordance with ISO 10272, *Yersinia enterocolitica* in accordance with ISO 10273, and *Escherichia coli* O157 in accordance with ISO 16654.

Annex C
(informative)

Comparison of procedures

C.1 Advantages of destructive methods

The excision of surface tissue harvests all bacteria on the surface while other methods do not. This invariably results in larger counts by the destructive method. Not all bacteria on the surface may be removed by either method or grow on the media and incubation conditions used.

Repeatability and reproducibility of destructive methods are less variable, because the sampling methods used in non-destructive testing result in greater operator variability.

C.2 Disadvantages of destructive methods

Only a small proportion of the carcass is sampled by destructive methods, which may result in significant inaccuracies when total contamination is low and heterogeneously distributed, or when the presence of the target pathogen is sparse.

Excision causes damage to the carcass, which may be commercially unacceptable.

C.3 Calculations of the diagnostic value

From the data of reference [3], it can be calculated that swabbing of pork carcasses recovers on average only 30 % of the number of *Enterobacteriaceae* that can be recovered by sampling a comparable surface area with the destructive method. Also the reproducibility of swabbing is poor and large variations in results are therefore common.

From the data of reference [4], it can be calculated for the detection of detecting beef carcasses contaminated with *Escherichia coli* or coliform microorganisms the sensitivity of swabbing a surface of 100 cm^2 is only 30 % to 40 % when compared to excising 100 cm^2. The calculated Kappa-value of 0,22 means that there is rather poor agreement between the results of both methods (Table C.1). Furthermore, Table C.2 demonstrates that at low levels of contamination even the destructive method is in practice not as consistently robust as might be expected. Table C.3 presents an approximation of the absolute sensitivity of swabbing and the destructive method. The estimated sensitivity of about 80 % for the destructive method (11/14) is always better than the estimated sensitivity of about 50 % for swabbing (7/14), but both techniques lead to an under-estimation of the true prevalence.

However, exact knowledge of the diagnostic value (i.e. the sensitivity, the specificity, the precision and the predictive value) of the still widely used (classical) sampling methods is not available in the literature (see reference [5]).

C.4 Sampling points

Sampling in the chill room 12 h to 24 h after slaughter may not be appropriate in all cases. Where flash chilling of pig carcasses at, for example, −30 °C to −35 °C is used, many pathogens are likely to be killed or sub-lethally injured, and hardening of the fat tissue makes it more difficult to recover bacteria.

Table C.1 — Swabbing compared with excision regarding the detection of carcasses with about 16 cfu coliforms per square centimetre

		Detected with excision (100 cm^2)		
		yes	no	Total
Detected with swabbing (100 cm^2)	yes	4	3	7
	no	7	16	23
	Total	11	19	30
Relative sensitivity (4/11)		36 %		
Relative specificity (16/19)		84 %		
Relative predictive value positive result (4/7)		57 %		
Relative predictive value negative result (16/23)		69 %		
Relative precision [(4+16)/30]		67 %		
Apparent prevalence (7/30)		23 %		
True prevalence [a] (11/30)		37 %		
Observed agreement between methods (20/30)		0,666		
Positive agreement (yes/yes) by chance [(7/30) × (11/30)]		0,086		
Negative agreement (no/no) by chance [(23/30) × (19/30)]		0,486		
Total agreement by chance (a)		0,572		
Observed agreement minus total agreement by chance (b)		0,094		
Maximum agreement outside of chance ($1–a$)		0,428		
Cohens' Kappa [b] [$b/(1–a)$]		0,220		

NOTE Calculated with data from reference [4].

[a] True prevalence as determined with excision.

[b] A Kappa value of between 0,4 and 0,7 is usual and represents fair to good agreement. A Kappa value of 0,22 represents poor agreement.

Table C.2 — Diagnostical value of swabbing and excision with regard to the detection of carcasses with about 16 cfu coliform organisms per square centimetre

		Are about 16 cfu coliforms/cm^2 actually present on carcasses?		
		yes	**no**	**Total**
Detected with excision (100 cm^2)	yes	11	0	11
	no	3	16	19
	Total	14	16	30
Detected with swabbing (100 cm^2)	yes	7	0	7
	no	7	16	23
	Total	14	16	30

NOTE Calculated with data from reference [4].