INTERNATIONAL STANDARD

ISO 13307

First edition
2013-03-01

Microbiology of food and animal feed — Primary production stage — Sampling techniques

Microbiologie des aliments — Stade de production primaire — Techniques de prélèvement

Reference number
ISO 13307:2013(E)

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Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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.

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Microbiology of food and animal feed — Primary production stage — Sampling techniques

1 Scope

This International Standard specifies sampling techniques within the primary food-animal production stage, for detection or enumeration of viable microorganisms with particular reference to food-borne pathogens.

This International Standard is not intended for use in diagnosis of animal disease.

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 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 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
primary production stage
includes all the stages of food production from farm until harvest or entry to the slaughterhouse

3.2
laboratory sample
sample prepared for sending to the laboratory and intended for inspection or testing

4 General arrangement

4.1 General

The parties concerned or their representatives may be given the opportunity to be present when sampling is performed.

Whenever special, e.g statutory, requirements are given for the sampling and/or arise from a specific analysis to be performed, these requirements shall be followed.

4.2 Sampling personnel

Sampling for microbiological examination shall always be undertaken by a person trained and experienced in the technique of sampling for microbiological purposes.

4.3 Packing and labelling of samples

Samples shall be packed in order to avoid cross-contamination and to prevent leakage or loss of moisture. They shall be clearly identified.

The minimum details that shall accompany the samples are: the nature of the matrix, its identification, the name or initials of the person responsible for taking the samples, as well as the date, time (if appropriate), and place of sampling.

This information should be recorded on a form. One form can be used for several samples provided each has unique identification and the samples are accompanied by the sampling form which lists the sample details with their unique identifying codes.

4.4 Preparation of a sampling form

Samples shall be accompanied by a report, ideally completed on a standard form provided by the laboratory, signed or initialled by the sampling personnel. The report shall give the following particulars:

— the place, date and time (if appropriate)of sampling;

— the names of the sampling personnel;

— the nature, number, and identity of samples constituting the consignment;

— the purpose of sampling and the microorganisms to be sought.

When appropriate, the report shall also include any relevant conditions or circumstances, and any special information relating to the product being sampled, e.g. difficulty in achieving representative samples.

If any additives such as diluents, transport media or neutralizing agents are used, these shall be recorded.

5 Diluents and disinfectants

5.1 Diluents.

5.1.1 General. Diluent used for moistening all kind of swabs (bootswabs, stick swabs etc.):

— peptone salt solution prepared according to ISO 6887-1;

— buffered peptone water prepared according to ISO 6887-1;

— sterile water;

— potable water for samples where this would not interfere with the analysis, e.g. bootswabs.

5.1.2 Medium for transporting swabs for specific purposes. The general aim of these media is to ensure survival of the target population, e.g. *Campylobacter* are particularly sensitive to drying.

Examples of transport media:

— buffered peptone water for *Salmonella* prepared according to ISO 6887-1;

— Cary–Blair transport medium or equivalent;

— Amies charcoal transport medium or equivalent.

In circumstances where the sample is acidic or alkaline, or may become so during transportation, it may be useful to use a buffered diluent.

Consideration should be given, if enumeration is intended, to the possibility of multiplication of the target or competing organisms before examination in the laboratory.

5.2 Disinfectants for decontamination of packaging, instruments and surfaces of certain samples

5.2.1 Ethanol 70 % volume fraction.

5.2.2 Alcohol wipes.

5.3 Neutralizers for disinfectant residues

5.3.1 General. An appropriate neutralizer for all situations cannot be prescribed as each disinfectant is optimally neutralized by a specific chemical substance (see Table 1). However, if the use of disinfectant is suspected, but its composition is unknown, a neutralizer for general use (5.3.2) can be used.

Table 1 — Neutralizing agent components and neutralized constituents

Neutralizing agent components	Neutralized constituents
Soya lecithin	Quaternary ammonium
Sorbitan monooleate (polysorbate 80)	Ethanol
L-Histidine	Aldehydes
Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	Halogen
	Phenols
Disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$)	Acid or alkaline

5.3.2 Neutralizer for general use.

5.3.2.1 Composition.

Soya lecithin	3,0 g
Sorbitan monooleate (polysorbate 80)	30,0 g
L-Histidine	1,0 g
Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)	7,8 g
Disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$)	100,8 g
Water	1 000 ml

5.3.2.2 Preparation. Dissolve the components in the water by heating. Sterilize for 15 min in the autoclave maintained at 121 °C. The prepared medium can be stored at (5°± 3) °C for 3 months in a tightly closed light-proof container.

This neutralizing liquid is normally used at 10 % volume fraction in diluents (5.1).

6 Apparatus and materials

6.1 Sampling equipment and description.

6.1.1 General. Non-disposable sampling equipment should be sterilized before use, e.g. by moist heat (autoclave) or dry heat (oven), according to ISO 7218. In certain situations, chemical decontamination may be appropriate. After such treatment, the equipment should be clean, sterile and free of inhibitory substances. If the equipment needs to be reused while taking samples, sterilization shall be done preferably by flaming (see 6.1.10) or with 70 % volume fraction ethanol or with any other appropriate disinfectant (refer to ISO 7218). Sealed packs containing multiple plastics disposable equipment (e.g. gloves, overboots, plastics bags) are suitable for use during sampling of products from the primary production stage. On each sampling occasion (e.g. each different farm), a fresh pack should be used. During sampling, every precaution shall be taken to avoid contamination of the unused disposable materials/equipment.

6.1.2 Gloves, disposable, single use, impervious, used during sampling to protect the sampler and to avoid cross-contamination. An alternative is to use plastics bags, inverted over the hand(s).

6.1.3 Overboots, strong clean plastics bags of appropriate size, or boot-shaped plastics covering made specifically to put over boots or shoes, used for two purposes: as biosecurity while visiting a farm to avoid introducing contamination; and to put over boots just before sampling with bootswabs (6.1.6).

6.1.4 Fabric swabs, normally large sterile portions of cloth, such as gauze, cellulose-based sponge, woven or non-woven fabric which are used to swab large surface areas.

6.1.5 Stick swabs, cotton-bud swabs and all swabs comprising small pieces of cotton or synthetic material fixed to the end of a wooden, metal or plastics stick. The swabs are often contained in sterile tubes which may contain medium such as Amies charcoal transport medium. The material used should be free of inhibitory substances unless these are specified for selecting the target agent.

6.1.6 Boot socks, also called **bootswabs** or **sock swabs**, adaptations of fabric swabs designed to be worn over the feet so that samplers can take the swabs while walking around doing other things. Boot socks used shall be sufficiently absorbent to soak up the moisture. They can be made from tubular elastic bandage material, which is cut into suitable lengths and pulled over the shoes or boots. Alternatively, commercial fabric overshoes (avoiding those with plastics soles) or other suitable and sterile material which covers the sole of the foot can be used, such as sterile fabric mob-caps (hair covers). In order to avoid possible contamination from the sampler's footwear, the boot socks should be put on, over new plastics overboots (6.1.3), after entering the area to be sampled.

6.1.7 Drag swabs, mostly used in the poultry industry, comprise a battery of four large moistened cloths (e.g. absorbent pads without antimicrobial substances) attached to a bar which is dragged over litter or slatted areas or pits containing faecal droppings. Small sponge drag swabs are also available commercially, but have a limited surface area compared to the original design.

6.1.8 Moore's drain swabs or **tampon swabs**, typically comprising large composite fabric swabs with multiple layers of gauze or cotton wool encased in gauze. Sanitary towels or tampons (free of antimicrobial substances), which are constructed in this way, are often used. Large cellulose sponges are also suitable.

6.1.9 Rope swab. A series of soft, sterile, manilla ropes of diameter 1 cm to 2 cm (e.g. seven ropes per large feedlot-type pen) placed horizontally just above the feed and water troughs so that cattle in the pen brush against them and are able to chew the ropes.

6.1.10 Portable gas burner or **blow torch**.

6.1.11 Sterile forceps, **scalpels**, **scissors**.

6.1.12 Sterile spoons or **spatulas**.

6.1.13 Sterile stiff brushes.

6.1.14 Cool box, insulated, either with integral cooling system, or cold packs, capable of maintaining the samples at low temperature (above 0 °C) during transportation to the laboratory.

6.2 Sample containers. Sample containers and closures shall be of materials and construction which adequately protect the sample and which do not bring about a change in the sample which could affect the results of subsequent analyses. These are usually plastics bags or rigid containers (plastics or glass screw-capped bottles or jars). Containers and closures shall be dry, clean, leak-proof and sterile.

The shape and capacity of the containers shall be appropriate to the particular requirements of the product to be sampled. Containers other than plastics bags shall be securely closed by means of suitable stoppers or secure caps.

7 Sampling techniques — General recommendations

Samples may be taken from animals and their environment, including during transportation and in the slaughterhouse, to monitor the carriage of zoonotic agents in the live animal.

Sampling shall be carried out in such a way as to obtain representative samples of the materials to be tested.

The samples shall be taken using aseptic techniques and equipment, materials, and containers specified in Clause 6.

The precise method of sampling and the mass or volume of matrix to be taken varies with the nature of the product and the purpose for which samples are required. For details of the requirements, see Clauses 8 to 11. The sample container shall be closed immediately after sampling.

8 Sampling techniques in the farm environment

8.1 Samples taken after cleaning and disinfection

Taking samples from disinfected surfaces is problematic because residual disinfectant can be present, and often the disinfectant used is not known. Specific or "universal" disinfectant neutralizers can be used, but some of these have an unpredictable effect on the growth of stressed organisms and competitive flora leading to false negative tests.

When sampling disinfected animal housing, it is best to sample after all surfaces have dried to minimize the inhibitory effect of disinfectant gathered with samples. Examples of places to sample are wall and floor surfaces, drinkers, feeders, nest boxes, partitions, movable equipment such as weighing machines, ventilation ducting, beams and ledges, control panels, and floors of anterooms or service areas. Conveyor systems passing through cage layer houses can also be sampled.

It is recommended, where practicable, to transfer the swab immediately after sampling into an excess (at least 1 part by mass to 100 parts by volume) of specific pre-enrichment or enrichment broth (e.g. a fabric swab into 225 ml BPW for *Salmonella* or other specific medium) which dilutes and/or inactivates the disinfectant. In this case, laboratory samples shall be cultured on the day of collection.

If same-day examination is not possible, diluents with neutralizers shall be used for moistening the swab before sampling.

If the disinfectant used is known, add the appropriate neutralizer (see Table 1) to the relevant diluent (5.1).

If the use of disinfectant is suspected but its identity is not known, add the "universal" neutralizer (5.3.2).

8.2 Surface sampling

8.2.1 Sampling with fabric swabs

This type of swab (6.1.4) can be held with a new glove (6.1.2) for each sample or using an "inverted bag technique", in which a polyethylene bag (6.1.2) holding the fabric swab is inverted to expose the swab, which is then used to sample the surface. Each swab is rubbed vigorously over selected surfaces so that each surface is covered and the swab is visibly soiled, sampling a minimum area of 1 m^2. The bag is then turned back the right way round, sealed, and used to transport the swab. When sampling dry surfaces, swabs should be moistened in a suitable diluent (5.1). Preferably both sides should be used to maximize the surface area swabbed and the recovery of material on the swab. Also the swab should be taken from multiple separate parts of the surface to be sampled.

When areas such as cracks and crevices are sampled, the fabric swabs can be folded over a sterile wooden spatula or similar instrument and forced down into the cracks with a slicing action, as if cutting a cake.

8.2.2 Sampling with stick swabs

To maximize recovery of organisms, stick swabs (6.1.5) should be as large as practicable.

When sampling from very moist sample areas they can be used dry, but if areas to be sampled are dry (such as environmental samples), the swabs should be moistened in a suitable dilution liquid (see 5.1).

Remove a swab from the sterile wrapping and moisten the tip by immersing it in a tube containing dilution liquid. Press the tip of the swab against the wall of the tube to remove excess fluid.

When sampling surfaces, a sufficiently large area should be swabbed to ensure that all surfaces of the swab are liberally coated with material. Ideally several different locations should be swabbed or multiple swabs taken to maximize recovery of the target organism. When sampling areas with cracks and crevices, aim to probe the full depths of organic material present and gather as much material as possible on the swab. After sampling, break or cut off the stick aseptically. Place in transport medium (see Clause 12) if required.

8.3 Sampling floors

8.3.1 Sampling with bootsocks

Ensure that the surface area of bootsocks (6.1.6) is maximized and that they are well wetted before use. Bootsocks should be worn over clean "overboots"(6.1.3) and neither the overboots nor the bootsocks should be allowed to come in contact with disinfectant footdips. It is therefore necessary to enter the area to be sampled through any footdip before putting on the overboots and bootsocks. Bootsocks can be used to sample any type of floor surface. Bootsocks that are used for sampling groups of animals should be taken before any change or replenishment of bedding.

The bootsocks may be moistened with potable water or other suitable diluent (see 5.1) or premoistened bootsocks can be used. It is important to walk over a large representative area of the space to be sampled, e.g. in poultry houses at least 100 steps should be taken per house, including the full length and width of the house and any littered or slatted areas and ensuring that areas of faecal accumulation or wet litter under drinkers are included.

Change overboots between separate epidemiological units.

8.3.2 Sampling with drag swabs

Drag swabs (6.1.7) can be used in the same situation as bootsocks and the same principles of sampling apply, i.e. it is essential to adequately represent the surfaces which are sampled, ideally using multiple swabs per area. In order to improve their efficiency, step on the drag swab at intervals, while wearing overboots (6.1.3).

8.3.3 Litter samples

8.3.3.1 Description

Litter is the faecally soiled bedding of animals.

8.3.3.2 Sampling procedure

Litter is a very convenient sample to take from floor-bedded poultry flocks, but is often taken in an unrepresentative way, in which case it may lack sensitivity. It is better to collect a large sample and then take test portions(s) on farm. The best way to take litter samples is to move over the whole house taking pinches of litter from at least 60 separate areas in the house to make up a final amount of about 2 kg. This sample can be sent to the laboratory or can be thoroughly mixed and a test portion of at least 25 g taken and sent to the laboratory. Litter samples should be taken before any replenishment of bedding.

8.3.4 Artificially pooled floor faecal samples

8.3.4.1 Description

Floor faeces are those previously voided by the animal, which are collected from the floor of the animal accommodation.

8.3.4.2 Sampling procedure

It is normally considered that 5 to 20 individual pieces of faeces can be pooled without drastically reducing the sensitivity of detection of *Salmonella* as a result of competition from competing flora and the action of inhibitory agents such as organic acids, bacteriocins or bacteriophage, but larger pools are often collected from poultry, mixed, and the test portion taken. This can increase the risk of diluting out and failing to detect positive material in the pool where the within-flock prevalence is low, but can also increase the chance of including faeces from a high-level shedder. Naturally mixed faeces such as those that accumulate on scrapers of manure belts or boards in poultry houses or on slurry ramps, scrapers or spreaders on pig and cattle farms are normally an excellent sample, as faeces from a large number of individual animals contribute to a sample that accumulates over time. It is essential to ensure that the distribution of samples taken is representative of the flock or herd. In large faecal samples, such as those from cattle, the target pathogen can be heterogeneously distributed within an individual faecal sample. Therefore, multiple test portions should be taken to maximize detection of target organisms.

For microorganisms for which culture-based detection tests are less sensitive, pooling of individual faecal samples is not recommended as the competitive effect of other flora is greater when the detection limit for the target organism is relatively high.

8.3.5 Naturally mixed faecal samples

8.3.5.1 Description

These are naturally merged faeces from animals kept in groups. Accumulation of faeces may occur over a period of time or be concentrated by the action of manure-cleaning operations or systems.

8.3.5.2 Sampling procedure

Naturally mixed faeces from individual pens or groups of animals, e.g. pigs or cattle, can be collected manually using spatulas, spoons (6.1.12) — sometimes incorporated in a sampling pot — or with a gloved hand. Alternatively, faeces can be gathered from the ground using a hand inside an inverted plastics bag (an inverted clean-side outwards plastics bag). After taking the faeces, the bag is removed from the hand, and turned right-side out to enclose the faeces. The bag can then be sealed. Alternatively, a fabric swab (6.1.4) may be used to sweep through areas where fresh faecal material has accumulated.