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

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

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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.

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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.

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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 ($Na_2S_2O_3 \bullet 5H_2O$)	Halogen
	Phenols
Disodium phosphate ($Na_2HPO_4 \bullet 12H_2O$)	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).

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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**.

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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).

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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).

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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.

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Examples of naturally mixed fresh faeces are mixed faecal accumulations which accumulate on droppings boards or scrapers after running scrapers or droppings belts in cage layer farms; material accumulated on yards or automated scrapers or manure ramps and manure spreaders in pig production; or manure in communal feeding or drinking areas in accommodation for dairy herds. There is no opportunity to take these types of samples in deep litter systems, but in this case there may be seepage of fluids from the litter which can be gathered with fabric swabs. Alternatively bootswabs can be used to sample bedding.

8.4 Dust samples

8.4.1 Description

Dust is the result of settlement of airborne particles derived largely from the dried faeces of animals, but also including material from the skin, hair or hide of the animals and desiccated environmental elements, e.g. dried and fragmented bedding, and fine feed particles.

Dust samples are not suitable for monitoring microorganisms sensitive to desiccation such as *Campylobacter*.

8.4.2 Sampling procedure

Dust can be collected manually using a gloved hand or from the floor using sterile suitable stiff brushes (6.1.13). Where dust is difficult to collect, scrapers can assist. Where dust is very sparse, moist fabric swabs (6.1.4) can be used to gather thin layers of dust from surfaces. As with most samples, the more dust that can be collected the more sensitive the test. It is best to avoid sampling dust from areas close to feed delivery systems, as feed dust particles are overrepresented in locally accumulated dust. Suitable places from which to collect dust are the baffles of air extractor fans, ledges, platforms and beams in the house structure or equipment/movable fittings, and corridors between pens of animals. It is possible to place temporary shelves in strategic places to accumulate dust for collection if the existing structure is unsuitable. Electrostatic devices can also be used. Dust should not be collected from space heaters or light fittings which have been used recently, as the heat and light reduce levels of bacteria in the sample.

8.5 Water samples

8.5.1 Description

Water samples should include sediment if present, and swabs from drinker systems.

8.5.2 Sampling procedure

Water can be satisfactorily sampled by using a dry fabric swab (6.1.4) to scoop up sediment from bell- or trough-type drinkers and swabbing around the interface between the trough and the water container, avoiding any algal or protozoal growth where possible. The swab also absorbs 20 ml to 30 ml of water during the operation.

It is normally not worth swabbing nipple drinkers or even their spillage cups in occupied housing, as this merely indicates general environmental contamination. For animals which drink from natural water sources at pasture, the best samples are sediments from slow-moving water at bends in the river or stream, or deep pockets in springs where such sediments accumulate. These sediments can be scooped up into a jar or gathered with a fabric swab.

When sampling the main piped water supply prior to delivery to the animals, it is best to gather large volumes (e.g. 5 l to 10 l) of water.

Another approach is to place an in-line filter in the water supply which can be tested periodically or to suspend a sterile cellulose sponge or tampon swab in the water tank, to which organisms preferentially attach. The swab can be removed and cultured after 1 day to 2 days *in situ*.

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8.6 Moore's drain swabs or tampon swab samples

Moore's drain swabs (6.1.8) are used to concentrate microbes in the fluids passing through liquid clearance systems such as drains or water courses.

These are useful screening samples for the detection of bacteria such as *Salmonella* on a site. They are typically anchored in drains or liquid waste handling passages for 5 days to 7 days, then removed and cultured.

8.7 Rope swabs

This method has been used successfully to detect verotoxin-producing *Escherichia coli* (VTEC) O157 in groups of cattle, but the same principle can apply to most environmentally robust microorganisms.

Cattle hide is normally contaminated by faeces and cattle frequently lick their hides during grooming which contaminates the tongue and oral cavity. If sterile ropes (6.1.9) are suspended just above feed and water troughs, the cattle in a pen rub against it and chew it, thereby transfering the bacteria to the rope. After approximately 24 h *in situ*, the ropes can be removed and cultured for the target microorganism by immersion in enrichment broth.

8.8 Disposable in-line milk filters

In-line milk filters are generally sited between the milking equipment and the bulk tank to remove large particles of foreign material, including faeces, from the milk. The filters are disposable and usually made of paper. Material from these provides a good overall assessment of the microbiological status and the standard of hygiene of the farm, because they gather material from various sources into a single sample.

If a disposable filter is not present, washings from static filters can be taken by rinsing the filter in diluent, which can then be used for various microbiological tests.

9 Techniques for taking samples from animals

9.1 Sampling from animals on the farm

9.1.1 Sampling with stick swabs

Animal restraint is often necessary. When taking rectal, cloacal or nasal swabs (6.1.5), take care to avoid injuring the animal while maximizing the recovery of material retrieved on the swabs. This is done by gently rotating the swab around its axis and at the same time carefully exploring the extremities of the cavity being sampled. Swabs with wooden sticks should not be used for rectal swabbing. The swab should be wetted before use with isotonic saline, sterile water or any medium favourable to bacteria and safe for the animal.

9.1.2 Faeces

9.1.2.1 Description

Fresh faeces should be taken either from the floor or directly from the rectum of the animal. It is usually easier to collect fresh faeces shortly after the animals have been fed, as feeding stimulates defecation.

9.1.2.2 Sampling procedure

A composite faecal sample consists of a mixture of fresh faecal matter picked up from at least five different places on the floor of the pen, which is representative of the distribution of faeces in the pen.

To sample faeces individually, restraint of the animal is often necessary. Faecal samples can be carefully extracted manually from the rectum using the fingers wearing new or sterile gloves (6.1.2) and changing gloves between each animal.

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9.1.3 Swabs from the skin, hide or feathers

9.1.3.1 Description

Skin, hide and feathers are frequently contaminated with faeces, and thus they are a convenient site to sample which can indicate the presence of pathogens in the group.

Large moistened swabs consisting of pieces of fabric are used (see 6.1.4).

9.1.3.2 Sampling procedure

Restraint of the animals can be necessary, but sampling can often be done during feeding when the animals' attention is distracted. Select a representative number of animals per pen.

Successively swab their backs or other contaminated areas (maximum five animals per swab). For pigs, swabbing the back from the shoulders to the tail with the same swab has been shown to be effective. For other types of animal, the perineal and ventral areas can be more suitable.

9.1.4 Recto-anal junction swab samples

9.1.4.1 Description

Various studies have demonstrated preferential colonization of VTEC O157 at the recto-anal junction in cattle. Sampling this manually by swabbing can therefore be more sensitive than sampling faeces from individual animals.

Gauze cloth swabs are used (6.1.4).

9.1.4.2 Sampling procedure

Individual animals are sampled manually by digital manipulation of a gauze cloth swab against the mucosa immediately inside the anus. In adult cattle, the swab can be wrapped around the hand and the hand rotated while performing a rectal examination.

9.2 Sampling animals at slaughter

9.2.1 Rectal content

9.2.1.1 Description

Rectal content collected at the slaughterhouse is taken after the death of the animal, by taking the rectum or rectum content after evisceration or in some cases it is possible to sample per rectum before evisceration.

9.2.1.2 Sampling procedure

Using a portable gas burner, flame the external surface of the rectum or disinfect with ethanol or alcohol wipes (5.2), allowing the disinfectant to evaporate.

Collect a minimum of 25 g of rectal content in sterile sealed bags or in sterile screw-capped containers.

9.2.2 Caecal content or whole caecum

9.2.2.1 Description

Caecal content is taken from the intestine after evisceration.

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In poultry, the best sample is the intact caeca. In pigs and other species, the blind end of the caecum can be tied off and sent to the laboratory, or the outside of the caecum can be carefully disinfected and contents sampled on site.

9.2.2.2 Sampling procedure

9.2.2.2.1 Poultry

The intestine proximal and distal to the caecum is transected by traction or cutting and both caecal sacs, still connected, are placed in a sterile container. An alternative is to carefully remove one full caecal sac without spilling the content.

9.2.2.2.2 Pigs and other animals

Take the intestine and block the caecal entry with one hand. Massage the contents of the caecum towards its blind end. Using a portable gas burner, flame the surface or disinfect with ethanol (5.2) which is allowed to evaporate.

Make a buttonhole-shaped incision in the caecum wall about 0,5 cm long using a sterile scalpel. Invert the caecum so that the buttonhole faces downwards. It is not usually necessary to exert any pressure on the caecum to expel the content through the buttonhole, but this can be done if necessary. Take care that the contents do not touch the non-sterilized part of the caecum wall and that no fluid drips from the outside of the intestine into the sample.

Collect the samples in bags or in large sterile containers with tightly screwed lids.

9.2.3 Mesenteric lymph nodes

9.2.3.1 Description

Isolate the gut. Identify the ileocaecal junction.

Pull apart the the ileo-caecal mesentery by hand to access the ileo-caecal lymph nodes.

9.2.3.2 Sampling procedure

Use new clean gloves (6.1.2) (ideally closely-fitting plastics gloves) and tear the nodes from the mesentery and surrounding adipose tissue manually. To achieve a 25 g pooled sample, it is usually necessary to include the proximal jejunal lymph nodes in the sample.

Place in sterile sealed bags or screw-capped containers.

9.2.4 Tonsils

9.2.4.1 Description

In some cases, it is necessary to remove the lower mandible from the head to access the pharynx. The lingual tonsils are paired at the root of the tongue; the palatine tonsil is in the palatine region (roof of the mouth) below the mucosa of the soft palate. The lingual tonsils are often removed with the pluck, or can remain attached to the head.

9.2.4.2 Sampling procedure

Using sterile tools (scalpel and forceps) (6.1.11), carefully dissect out the two lingual tonsils avoiding cutting the tonsils themselves. If the lingual tonsils are absent, take the palatine tonsil. Place in a closed sterile bag or sterile screw-capped container.

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10 Sampling techniques in the hatchery

10.1 General

This type of sampling is intended mainly for the detection of *Salmonella.*

10.2 Hatcher basket liner samples

10.2.1 Description

Hatcher basket liners are sheets of paper which are used to line plastics or metal trays in which eggs are hatched.

10.2.2 Sampling procedure

Sample as large a surface area of liner as possible, totalling about 1 m^2, which means that the resulting large bulky samples have to be cultured in large bags or containers to accommodate them. Where possible, also include broken eggshells, these can be included in the sample and crushed down within the liner. Liner samples should be carefully gathered into sterile bags so as to retain as much adherent material as possible.

10.3 Broken eggshells

10.3.1 Description

These types of samples are the empty shells remaining after hatching and removal of chicks.

10.3.2 Sampling procedure

Broken shells from hatched eggs can be collected from systems where basket liners are not used. Egg fragments from as many baskets as possible per hatcher should be collected in a large bag.

10.4 Hatcher fluff

10.4.1 Description

This sample is light dusty material largely derived from the integument of chicks, together with material from dried meconium and some dust from the surfaces of the hatcher.

10.4.2 Sampling procedure

Although there is typically a large amount of fluff released during hatching, some material is more suitable for testing than others. Fluff from the floor of the hatcher is usually best, but when this is very wet due to high humidity in the hatcher and has to be posted to the laboratory, it is sometimes better to concentrate on samples of drier fluff from higher fittings, particularly that which has accumulated in the air extractor ducting of the incubator. This can be gathered using a gloved hand or with a fabric swab.

To avoid handling fluff in the laboratory and the consequent risk of cross-contamination, it is advisable to collect the amount of fluff to be analysed in sufficiently large bags or vessels so that culture medium can be added directly to the collection vessel. The required quantity of fluff or dust can also be placed in a loose gauze pouch to minimize dispersal of fine particles during laboratory manipulations.

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10.5 Meconium

10.5.1 Description

These are samples of early intestinal output of newly hatched chicks gathered by manual expression of the cloaca.

10.5.2 Sampling procedure

This operation is normally carried out during sexing when the cloaca has to be manually examined as part of the normal chick processing operation. Meconium is expressed into a container until a pool of samples from at least 250 chicks has been obtained. Preferably this should be at least 300 so that a 1 % prevalence of meconium contamination can reliably be detected.

10.6 Swabs from hatcher baskets

10.6.1 Description

These are taken with fabric swabs (6.1.4).

10.6.2 Sampling procedure

See 8.2.1.

10.7 Macerated hatchery waste

10.7.1 Description

This sample includes all the material originating from the hatch of a batch of eggs: eggshells; dead-in-shell embryos; dead chicks; and culls which are processed through a macerator machine before discharge into a waste skip.

10.7.2 Sampling procedure

Fabric swabs from the interior, spillage and splashed material from the macerator machine can be used to assess contamination of previously macerated material.

10.8 Dead-in-shell embryos

10.8.1 Description

These are embryos of chicks which have failed to hatch and which are subject to post-mortem examination to detect vertically transmitted *Salmonella*.

Since the eggs are still intact, the embryos are a fairly specific indicator of likely *Salmonella* infection in breeding flocks. The problem is that the frequency of internal egg-borne transmission is normally very low, so unless large numbers of samples are examined the sensitivity of detection of infection is low. This sample also does not take into account transmission of *Salmonella* via contamination of shell surfaces, cracks, and shell membranes.

10.8.2 Sampling procedure

Usually, a minimum of 60 eggs is taken and brought to the laboratory. Samples of internal organs and yolk sacs are taken aseptically from the embryos and pooled.

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10.9 Cull chicks

10.9.1 Description

These are live hatched chicks which are considered by virtue of sex (males from egg-laying lines) or physical defects to be unsuitable for rearing and which are normally culled at the hatchery.

10.9.2 Sampling procedure

The examination is carried out according to similar principles as the dead-in-shell embryo samples. Usually, a minimum of 60 chicks is taken and brought to the laboratory.

10.10 Samples from chick deliveries on the farm

10.10.1 Description

This sample is a sheet of paper which is put on the bottom of the chick boxes during their transport to the farm. The chicks soil the papers with their meconium. When wood-wool is used as a liner, this is a less suitable sample due to its bulk and it may be better to sample debris from the bottom of numerous boxes using fabric swabs.

10.10.2 Sampling procedure

This sample should preferably be taken before the chicks enter the house, but this may not always be practical. Samples should be taken with great care to minimize contamination from the house or sampler's hands.

Put at least five basket liners per flock, total surface area at least 1 m^2, in a sterile plastics bag and put this sample in a second bag before sending it to the laboratory.

11 Techniques for sampling animal transport vehicles, modules, and crates

11.1 General

Swabbing of animal transport should be carried out as soon as possible after removal of the animals.

11.2 Vehicles for large animals (cattle, sheep, pigs, horses)

Large hand-held fabric swabs (6.1.4) or, where appropriate (in cattle lorries), bootswabs (6.1.6) can be used to sample residual faecal material on floor surfaces. Sampling should include all subsections of the flooring in a representative way. As with any surface sampling, the larger the surface sampled and the higher the number of separate samples taken and tested, the greater the likelihood of detecting the target organism.

11.3 Transport crates and modules for poultry

Effective cleaning and disinfection of crates used for delivery of meat birds to slaughter is problematic, because contamination detected by swabbing can relate to a previous rather than the current batch of birds. Despite this, swabbing of faecal residues in crates and on the floor of the transporting vehicle can be useful to assess the possibility of contamination of the slaughter-batch. As with any surface sampling, the higher the number of crates swabbed in a representative way throughout the batch, the greater the chance of detection of organisms. Only the floor of the crates should be swabbed, and it is better to sample a large number of crates quickly than a small number more comprehensively.

In the case of crates and modules used for transporting birds between rearing and finishing or laying (breeding or commercial egg production) sites the equipment should be effectively decontaminated between batches so swabbing should be more representative of the status of the current batch.

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11.4 Sampling transport vehicles after cleaning and disinfection

Hand-held fabric swabs (6.1.4) should be used to sample as comprehensively as possible the surfaces that are exposed to the animals, as well as general surfaces within the vehicle including any ventilators.

To assess the potential for vehicles to (still) carry contamination (after cleaning and disinfection), footwells, wheel arches, and surface material can be swabbed.

12 Storage and transport of samples

12.1 General recommendations

For general requirements, refer to ISO 7218.

Transport samples according to international biosecurity regulations.

The delay between sampling and testing should be as short as possible. The samples should preferably be cooled before being put into insulated transport containers, and transport should be conducted chilled. Samples transported at ambient temperature should preferably be examined within 24 h of collection. Samples chilled during transportation should be examined within 72 h.

For faecal and environmental samples for *Salmonella* detection, transportation can be at ambient temperature as long as excessive heat (over 25 °C) and exposure to sunlight are avoided, and they are examined within 72 h.

12.2 Recommendations for sensitive organisms

For sensitive microorganisms, such as *Campylobacter*, all swabs should be moist and transported and tested within 24 h at ambient temperature, and within 48 h or 72 h, depending on the sample type (see Table 2) if chilled. Precaution should be taken to avoid freezing of chilled samples. In some cases, a transport medium is useful, e.g. Amies transport medium for stick swabs or Cary–Blair medium for bootsocks (5.1.2). Laboratory samples that cannot be tested on the day of arrival shall be kept refrigerated until analysis.

Recommendations for different types of samples taken for sensitive microorganisms are listed in Table 2.

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Table 2 — Recommendation for storage, transport, temperature and time for sensitive microorganisms

Sample type	Transport medium	Temperature	Maximum time before examination
Faeces (choose fresh and moist samples)	Not necessary (sample naturally moist)	2 °C to 10 °C	72 h
		Ambient temperature 2 °C to 25 °C	24 h
Boot socks Environmental swabs (moistened before use)	Keep moist by adding adequate transport medium or collect into the container of medium to be used for culture	2 °C to 10 °C	48 h Or the day of collection if the sample is collected in the whole volume used for enrichment
		Ambient temperature 2 °C to 25 °C	24 h
Caecal content (only for detection)	Not necessary if intact caeca (sample naturally moist)	2 °C to 10 °C	72 h
		Ambient temperature 2 °C to 25 °C	48 h
Caecal content for enumeration	Not necessary if intact caeca (sample naturally moist)	2 °C to 10 °C	48 h
		Ambient temperature 2 °C to 25 °C	24 h
Stick swabs of rectal, cloacal or caecal contents or fresh faeces	Amies medium	2 °C to 10 °C	72 h
		Ambient temperature 2 °C to 25 °C	48 h

Also see the specific standards for the microorganisms under study for any additional requirements on storage and transport.

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