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Microbiology of food and animal feeding stuffs — General rules for microbiological examinations

Microbiologie des aliments — Règles générales pour les examens microbiologiques

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Contents

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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.

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.

International Standard ISO 7218 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 7218:1985), which has been technically revised.

Annexes A and B form an integral part of this International Standard. Annex C is for information only.

Introduction

When conducting microbiological examinations, it is especially important

- that only those microorganisms which are present in the samples are isolated or enumerated, and

- that the microorganisms do not contaminate the environment.

In order to achieve this, it is necessary to pay attention to personal hygiene and to use working techniques which ensure, as far as possible, exclusion of extraneous contamination (see clause 5).

Since, in this International Standard, it is possible to give only a few examples of the precautions to be taken during microbiological examinations, a thorough knowledge of the microbiological techniques and of the microorganisms involved is essential. It is important that the analyses be conducted as accurately as possible, including calculation of the number of microorganisms and the variability of the results (part of this is given by the confidence limits; see clause 9).

Ultimately, it is the responsibility of the head of the laboratory to judge whether the manipulations are safe and can be considered to be good laboratory practice.

A large number of manipulations can, for example, unintentionally lead to cross-contamination and the analyst should always verify the accuracy of the results given by his or her technique.

In order to conduct the examinations correctly, it is necessary to take certain precautions when constructing and equipping the laboratory (see clause 3).

Certain precautions must be taken, not only for reasons of hygiene, but also to ensure good reproducibility of the results. It is not possible to specify all the precautions to be taken in all circumstances, but this International Standard at least provides the main measures to be taken when preparing, sterilizing and storing the media and the equipment (see clauses 6 and 7).

If the guidance given in this International Standard is followed, this will also contribute towards the protection of the health of the personnel. Additional information on this subject is to be found in the literature listed in annex C.

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Microbiology of food and animal feeding stuffs — General rules for microbiological examinations

1 Scope

This International Standard gives general instructions for carrying out microbiological examinations in accordance with specific standards.

The purpose of this International Standard is to help to ensure the validity of the examinations, to ascertain that the general techniques used for conducting these examinations are the same in all laboratories, to help achieve homogeneous results in different laboratories, and to contribute towards the protection of the health of the laboratory personnel by preventing risks of infection.

This International Standard may be used wholly or partly for the accreditation of a laboratory by national organizations.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6887:1983, *Microbiology - General guidance for the preparation of dilutions for microbiological examination*

3 Premises

3.1 Test areas

The areas required for the specific operation of a microbiology laboratory are as follows:

- receipt, storage, preparation and processing of the samples;

- preparation and sterilization of culture media and equipment;

- performance of analyses: weighing, dilutions, inoculations, subculturing, incubation, preservation of the strains, etc.;

- decontamination and cleaning of equipment, and processing of the analysis waste.

3.2 Additional areas

The areas included in this category are, for example:

- entrances, corridors, stairways, goods lifts or lifts;
- administrative areas (e.g. secretarial, offices, documentation rooms, etc.);
- cloakrooms and toilets;
- archive rooms;
- stores.

3.3 Location of the premises

The environment within which the microbiological analyses are carried out shall not affect the reliability of the analyses.

Care shall be taken to locate the premises so as to avoid risk of cross-contamination. Application of the "no-way-back" principle may help to achieve this aim.

Care shall be taken to ensure protection against extreme conditions such as excess temperature, dust, humidity, steam, noise, vibration, exposure to direct sunlight, etc.

The surface area shall be sufficiently large to keep the work areas clean and orderly. For all test premises, a work station of approximately 20 m^2 is recommended for each analyst.

During the course of the tests, care shall be taken to limit access to the test areas to only those persons required to conduct the tests.

Separate rooms and/or separate areas and/or specific enclosures shall be provided for the following:

- receipt and storage of samples;
- preparation of samples, particularly in the case of raw materials (e.g. powdered products containing a high number of microorganisms);
- manipulation of pathogens (e.g. *Salmonella, Listeria monocytogenes)*;
- preparation and sterilization of culture media and equipment;
- cleaning of glassware and of other equipment, as well as the decontamination of equipment and contaminated culture media;
- checking the sterility of foodstuffs.

Separation of the following areas should also be considered:

- the areas used for the preparation of culture media, and the room used for sterilization of culture media and of the equipment; and
- the decontamination area and washing area.

Incubators, refrigerators and freezers can be placed in specific, specially adapted rooms.

3.4 Equipping the premises

3.4.1 The test premises shall be fitted out in the following ways in order to reduce the risks of contamination by dust and therefore by microorganisms:

- the walls, ceilings and floors should be smooth, easy to clean, and resistant to detergents and disinfectants used in laboratories;

- overhead pipes conveying fluids should not cross the premises unless they are hermetically enclosed;

- solar radiation protection systems shall be installed on the outside of the windows, except in special cases;

- windows and doors shall be able to be closed hermetically when conducting the tests in order to minimize draughts; furthermore, they shall be designed so as to avoid the formation of dust traps and thus facilitate their cleaning.

3.4.2 The ambient temperature and air quality (microorganism content, humidity, dust spreading rate, etc.) shall be compatible with carrying out the tests. A filter ventilation system for incoming air is recommended for this purpose.

When tests are to be conducted in a low-contamination atmosphere, the room shall be specially equipped with a clean air laminar-flow cabinet and/or a safety cabinet.

This equipment shall comply with the relevant regulations.

3.4.3 The laboratory bench tops and furniture shall be manufactured in smooth, impermeable material, which is easy to clean and disinfect. In order to prevent the accumulation of dust, the cupboards shall, if possible, reach up to the ceiling.

Laboratory furniture shall be designed so as to facilitate cleaning the floors (e.g. movable furniture).

Enclosed storage facilities shall be available for storing documents used when manipulating the samples, culture media, reagents, etc.

NOTE – It is desirable that documents or books which are not frequently used be placed outside the test areas.

3.4.4 The premises shall be well lit with avoidance of interfering reflections. It is advisable to avoid as far as possible exposure of the work places and sensitive equipment (incubators in particular) to direct sunlight.

3.5 Maintenance and inspection

The floors, walls, ceilings, laboratory bench tops and furniture shall be subjected to regular maintenance and repair in order to avoid cracks where dirt might particularly accumulate and thus cause a source of contamination.

Regular cleaning and disinfection shall be carried out in order to keep the premises in a condition suitable for conducting tests.

The ventilation systems and their filters shall be regularly maintained and filters changed when necessary.

The microbiological quality of surfaces and air shall be monitored regularly.

Surface contamination may be estimated by directly applying to the surface a contact plate containing suitable neutralizing agents. The air quality may be examined by exposing for 15 min an open Petri dish containing a non-selective agar culture medium [e.g. plate count agar (PCA)].

NOTE – Other methods can also be used in order to estimate surface and air contamination.

4 Installations and equipment

In general, all installations and equipment shall be kept clean and in proper working condition. Maintenance operations should be monitored. The monitoring instruments shall be regularly serviced.

4.1 Microbiological cabinets

4.1.1 Description

A cabinet is a dust-removed work station equipped with horizontal or vertical laminar air-flow. In microbiology, a safety cabinet is used to retain the microorganisms on filters.

Conventionally, the maximum tolerable number of particles per cubic metre with a size greater than 0,5 µm represents the dust-spreading class of a safety cabinet. For cabinets used in food microbiology, the number of particles shall not exceed 4 000 per cubic metre.

Cabinets are of two types:

a) clean-air cabinets, which are intended to protect the product from extraneous contamination, and to minimize contamination due to the operator;

b) safety cabinets, which are intended to protect the product from extraneous contamination, and also to protect the operator and the environment.

Safety cabinets should be used for all work involving pathogens.

4.1.2 Maintenance and inspection

The efficiency of a safety cabinet shall be checked on receipt and thereafter at regular intervals by a qualified person (a yearly inspection is recommended). In the case of cabinets with prefilters, the latter shall be changed regularly.

Cabinets should be cleaned and disinfected after use. Periodic verification of any microbial contamination should be carried out by a check of the working surface and walls of the cabinet.

A periodic verification of the proportion of microorganisms present shall be carried out using the usual equipment. For example, expose several open Petri dishes containing a non-selective agar culture medium (e.g. PCA) in each cabinet for 30 min. Other methods can also be used.

4.2 Balance

4.2.1 Use

A food microbiology laboratory shall be equipped with balances of the required range and accuracy for the different products to be weighed. In general, two degrees of accuracy are required: ± 0,01 g and ± 0,0001 g.

These balances are mainly used for weighing the test portion of the sample to be analysed and the components of the culture media and reagents. They may also possibly be used for carrying out measurements of dilution fluid volumes by weight.

4.2.2 Maintenance and inspection

The balance shall be placed on a stable horizontal support and shall be protected from vibrations.

It shall be checked regularly by calibration with working standards (preferably each working day). At least once a year, its entire range shall be monitored by a qualified person.

The balance pan shall be cleaned, if necessary, after each use and at least once a day. The mechanism shall be cleaned and checked by a qualified service engineer at least once a year.

4.3 Homogenizer

4.3.1 Description

This equipment is used to prepare the initial suspension from the test sample of non-liquid products.

The following apparatus may be used:

- a peristaltic homogenizer with sterile plastic bags, possibly with a device for adjusting velocity and time; or

- a rotary homogenizer, the rotational speed of which is between 8 000 r/min and 45 000 r/min inclusive, with sterilizable glass or metal bowls equipped with covers.

In certain special cases, the homogenization can be carried out with sterilizable glass beads having an appropriate diameter (approximately 6 mm; see specific standards).

4.3.2 Use

The usual operating time of a peristaltic homogenizer is 1 min to 2 min. This type of apparatus shall not be used for certain foodstuffs, such as

- products which risk puncturing the bag (presence of sharp, hard or dry particles), or

- products which are difficult to homogenize because of their texture (e.g. salami-type sausage).

The rotary homogenizer shall operate for a duration such that the number of revolutions is between 15 000 and 20 000 inclusive. Even with the slowest homogenizer, this time shall not exceed 2,5 min.

Glass beads can be used for the preparation, by shaking, of the initial suspensions of certain viscous or thick products, in particular certain dairy products (see specific standards).

4.3.3 Maintenance and inspection

The different appliances shall be inspected and maintained in accordance with the manufacturers' instructions.

4.4 pH-meter

4.4.1 Description

A pH-meter is used to measure the potential difference, at a determined temperature, between a measuring electrode and a reference one, both electrodes being introduced into the product. It shall be capable of measuring to an accuracy of ± 0,1 pH units and its minimum measuring threshold shall be 0,01 pH units. The pH-meter shall be equipped with either manual or automatic temperature equalization.

NOTE – The measuring electrode and the reference electrode are usually grouped together in a combined electrode system.

4.4.2 Use

A pH-meter is used to measure the pH of each batch of culture media and reagents (7.2) to check if adjustment is needed. It may also be used to measure and/or adjust the pH of the test sample or of the initial suspension. Use of a pH-meter will be discussed in the standard specific to the product to be analysed, in which the conditions for the determination of the pH, for the adjustment of the pH, as well as the method of cleaning and of decontamination of the electrodes will be specified.

4.4.3 Maintenance and inspection

The pH-meter shall be calibrated, in accordance with the manufacturer's instructions, using at least two standard buffer solutions, at least daily before use. The standard solutions have pH values which are known to be within the second decimal at the measurement temperature (in general, pH 4,00 and pH 7,00 at 20 °C). They shall encompass the measured pH values.

The electrodes shall be checked and maintained in accordance with the manufacturer's instructions. It is necessary, in particular, to monitor regularly:

- the condition of the electrodes with respect to ageing and soiling;

- the response time and stability.

Prior to each use, check that the measuring bulb of the electrodes is completely immersed in distilled water or any other liquid, as recommended by the manufacturer; otherwise, immerse it 24 h prior to conducting any measurements.

Clean the electrodes after each use. In order to take into account the soiling and ageing of the electrodes, regularly clean them more thoroughly in accordance with the manufacturer's instructions.

Store the electrodes in accordance with the manufacturer's instructions.

4.5 Autoclave

4.5.1 Description

An autoclave is an appliance which enables a saturated steam temperature of at least 121 °C to be attained with a view to the destruction of microorganisms.

4.5.2 Use

During the same sterilization cycle, the autoclave shall not be used to sterilize clean equipment (and/or culture media) and also to decontaminate used equipment (and/or used culture media). It is preferable to use separate autoclaves for these two processes.

The autoclave shall be equipped with:

- at least one safety valve;

- a pressure gauge;

- a drain cock;

- a regulation device allowing the temperature to be maintained to within ± 1 °C of the scheduled value;

- a thermometer or a recording thermocouple.

It should preferably also be equipped with a duration indicator or a programmer/timer.

With steam sterilization all air must be expelled prior to the pressure build-up. If the autoclave is not fitted with an automatic evacuation device, it is necessary to remove the air until a continuous jet of steam is emitted.

4.5.3 Maintenance and inspection

The autoclave shall be kept in perfect operating condition and shall be regularly inspected by the competent departments in accordance with the manufacturer's instructions.

All the monitoring instruments shall be kept in perfect working order and shall be verified regularly.

Descaling, if necessary, and draining operations shall be carried out regularly.

4.6 Incubator

4.6.1 Description

An incubator consists of a chamber which enables a temperature to be kept stable and evenly distributed to within ± 1 °C, unless otherwise stated.

4.6.2 Use

Incubators shall be equipped with a regulation system which allows the temperature to be kept even and stable over their entire working volume.

If the ambient temperature is close to or higher than that of the incubator, it is necessary to fit a cooling system to the chamber.

Incubators walls should be protected from direct sunlight.

If possible, incubators should not be completely filled in one single operation because the culture media will take a long time to equilibrate to temperature, whatever type of incubator is used (forced-air convection or otherwise).

When loading incubators, attention should be paid to air circulation; under no circumstances shall Petri dishes or tubes be placed within 25 mm of the inside walls of the incubator. Stacks shall not be of more than six Petri dishes and shall be separated by at least 25 mm.

4.6.3 Maintenance and inspection

The homogeneous temperature within the working volume shall be checked using several thermometers or thermocouples.

The measurement accuracy should be four times better than the requested accuracy (e.g. for a requested accuracy of ± 2 °C, the measurement accuracy should be ± 0,5 °C).

The temperature stability shall be checked, for example, with one or more maximum and minimum thermometers.

The incubator temperature shall be checked at least every working day. For this purpose, each incubator shall incorporate at least one thermometer, whose bulb is immersed in glycerol contained in a sealed bottle. Other checking systems of equivalent performance can be used.

The inner and outer walls of the incubator shall be regularly cleaned and disinfected and, if appropriate, dust shall be removed from the ventilation system.

4.7 Refrigerator or cold-storage room

4.7.1 Description

These are chambers which allow cold storage to be guaranteed. The temperature, unless otherwise specified, shall be + 3 °C ± 2 °C except for the conservation of analysis samples where the temperature shall be + 2 °C ± 2 °C.

4.7.2 Use

Different chambers shall be available for the storage of:

- non-inoculated culture media and reagents;
- samples for analysis;
- microorganism strains and incubated media.

The refrigerators and cold-storage rooms shall be loaded in such a way that appropriate air circulation is maintained.

4.7.3 Maintenance and inspection

The temperature of each chamber shall be checked each working day using a thermometer or a permanently installed probe. (See 4.6.3 for the accuracy of the apparatus).

The following maintenance operations shall be carried out regularly:

- removal of dust from the blades or from the external heat-exchange plates;
- defrosting;
- cleaning and disinfection of the inside of the chambers.

4.8 Freezer

4.8.1 Description

A freezer has chambers which allow frozen storage to be guaranteed. The temperature, unless otherwise specified, shall be below –18 °C, preferably equal to –24 °C ± 2 °C.

4.8.2 Use

Different chambers shall be available for the storage of:

- some non-inoculated culture media and reagents;
- samples for analysis;