INTERNATIONAL STANDARD

ISO 8607

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Artificial insemination of animals — Frozen semen of breeding bulls — Enumeration of living aerobic microorganisms

Insémination artificielle des animaux — Semences congelées de taureaux reproducteurs — Dénombrement des micro-organismes aérobies vivants

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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.

ISO 8607 was prepared by Technical Committee ISO/TC 34, *Food products*.

This first edition of ISO 8607 cancels and replaces ISO/TR 8607:1991, which has been technically revised.

Introduction

The quantitative microbiological control of the hygienic collection and handling of bovine semen is of great importance in order to predict the efficiency of artificial insemination and to fulfil the requirements of biosecurity (see reference [1]). For the same reason, the investigation of bacterial contamination and the possible presence of facultative pathogenic microorganisms in the preserved bovine semen is also very important.

There is a need for an international method suitable for the determination of the microbial count in frozen semen, which indicates the hygienic status during collection, handling and storage. The aim of the colony-count method specified in this International Standard is to enumerate the saprophytic microorganisms that are originally present in and/or are transmitted to the bovine semen from the environment. With this method only the total count of bacteria is detected, mainly the aerophilic and mesophilic saprophytic ones, as well as a few facultative pathogenic microorganisms that are not very sensitive to environmental conditions.

Since samples of frozen bovine semen contain additional antibiotics, the determination of microbiological contamination of this type of sample is slightly different from the commonly used microbiological methods. When examining preserved semen samples in low dilutions, the number of colonies may be lower than expected and do not follow the usual proportions. Therefore relatively high decimal dilutions should be used to compensate for the inhibition effect of the antibiotics. As a result of the necessary high dilutions, 15 or less colonies can be observed in each Petri dish and this result should be accepted. This differs from the usual microbiological examinations of food where the sample dilution can be chosen in such a way that the number of colonies is more than 15 in a Petri dish so more precise examination is possible.

Microbial cells often occur as clumps or groups in the samples. Whereas shaking samples and dilutions may uniformly distribute the clumps of bacteria, this may not completely disrupt the clumps themselves into single cells. Consequently, each colony that appears on the medium can arise from a clump of cells or from a single cell and therefore it is more precise to express the result as the number of colony-forming units (CFU) of microorganisms than to give the number of microorganisms (see reference [2]).

This International Standard does not specify a tolerable limit value for the total CFU of bacteria, which may be a consumer requirement in trade. This should be given in commercial contracts.

A list of publications related to this International Standard is given in the Bibliography.

Artificial insemination of animals — Frozen semen of breeding bulls — Enumeration of living aerobic microorganisms

1 Scope

This International Standard specifies a method for the enumeration of living aerobic microorganisms present in the frozen semen of breeding bulls. The colonies growing in a solid medium after aerobic incubation at 37 °C are counted. The microbiological contamination of the sample is expressed as a number of colony-forming units of microorganisms per millilitre of the test sample.

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 rules for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6887-1 and the following apply.

3.1
semen
product of the genital organs of a male, intended for the fertilization of a female

3.2
ejaculate
quantity of semen obtained as a result of mating the male

3.3
dose
quantity of semen which is packaged individually and carries a unique identification, intended for a single artificial insemination

3.4
series of doses
group of doses of semen obtained from one bull and prepared from one or more ejaculates, obtained on the same day and subjected to the same treatment

3.5
living aerobic microorganisms
bacteria, yeasts and moulds which grow aerobically at 37 °C under the conditions specified in this International Standard

3.6
colony-forming unit
CFU
single microbial cell, or clumps or a group of cells, forming one colony on the medium under the conditions specified in this International Standard

4 Principle

Two poured plates are prepared using a specified culture medium. These are deep inoculated with a specified quantity of test sample, followed by aerobic incubation at 37 °C.

The number of CFU of microorganisms per millilitre of the test sample is calculated from the number of colonies obtained.

5 Diluent and culture medium

For general guidance, see ISO 7218.

Chemical products shall be of recognized analytical quality and suitable for microbiological analysis.

The water used shall be distilled water or of equivalent quality (see ISO 7218).

5.1 Diluent

The diluent is a peptone salt solution as specified in ISO 6887-1. Its composition, preparation and use are given only for the convenience of the users of this International Standard.

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation should be used. The manufacturer's instructions shall be rigorously followed.

5.1.1 Composition

Enzymatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

5.1.2 Preparation

Dissolve the components in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.1.3 Distribution and sterilization

Dispense the diluent in volumes as necessary for the preparation of the initial suspensions into test tubes or flasks (6.3) of appropriate capacity.

Dispense the diluent in volumes as necessary for the preparation of the decimal dilutions into test tubes or flasks (6.3) in quantities such that, after sterilization, each tube or flask contains 9,0 ml. The uncertainty of measurement of this final volume, after sterilization, shall not exceed ± 2 %.

5.2 Agar medium

5.2.1 Composition

Meat extract	10,0 g
Anhydrous D-glucose ($C_6H_{12}O_6$)	1,0 g
Dehydrated yeast extract	2,5 g
Peptone	3,0 g
Sodium chloride (NaCl)	2,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	2,0 g
Gelatine	10,0 g
Agar in powder or flake form	12,0 g to 18,0 g[1)]
Water	1 000 ml

5.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2 at 25 °C.

Dispense the medium into tubes or flasks (6.3), in quantities such that the container is half-full.

Sterilize in an autoclave (6.1) at 121 °C ± 1 °C for 15 min.

If the medium is to be used immediately, cool it to 44 °C to 47 °C in the water bath (6.8) and then add 10 % (by volume) inactivated and sterilized[2)] bovine or sheep serum. Otherwise, before beginning the microbiological examination, completely melt the medium in the boiling water bath (6.9), cool to 44 °C to 47 °C in another water bath (6.8), and then add 10 % (by volume) inactivated and sterilized[2)] bovine or sheep serum.

6 Apparatus

NOTE Disposable apparatus is an acceptable alternative to reusable glassware, if it has suitable specifications.

Usual microbiological laboratory apparatus and, in particular, the following.

6.1 Sterilizing oven (for dry sterilization) or **autoclave** (for wet sterilization), see ISO 7218.

6.2 Incubator, capable of being maintained at 37 °C ± 1 °C.

6.3 Test tubes, of 16 mm diameter and 160 mm length, or **flasks**, of capacity not greater than 500 ml.

6.4 Petri dishes, made of glass or plastic, of 90 mm to 100 mm diameter.

6.5 Pipettes, having a nominal capacity of 1 ml, graduated in 0,1 ml divisions.

Blow-out pipettes shall not be used.

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1) According to the gel strength of the agar.

2) By ultrafiltration (0,2 nm filter).