EAST AFRICAN COMMUNITY

EDICT OF GOVERNMENT

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EAS 217-2 (2008) (English): Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Part 2: Colony count technique at 30o C

ISO INSIDE

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EAST AFRICAN STANDARD

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Part 2: Colony count technique at 30^{o} C

EAST AFRICAN COMMUNITY

First Edition 2008

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that “Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose”.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

This East African Standard has been revised and aligned to ISO 4833:2003, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 °C*

INTERNATIONAL STANDARD

ISO 4833

Third edition
2003-02-01

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

Microbiologie des aliments — Méthode horizontale pour le dénombrement des micro-organismes — Technique de comptage des colonies à 30 °C

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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 4833 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition cancels and replaces the second edition (ISO 4833:1991). The following technical changes have been made:

— subclause 5.2, Plate count agar: an examination of dairy products is included;

— Clause 10, Expression of results: precision data are given, and an example of precision data for dairy products.

Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from them in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with this horizontal method. In cases where International Standards already exist for the product to be tested, they should be followed. It is hoped that when such standards are reviewed, they will be changed to comply with this International Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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

1 Scope

This International Standard specifies a horizontal method for the enumeration of microorganisms, by counting the colonies growing in a solid medium after aerobic incubation at 30 °C. Subject to the limitations discussed in the introduction, this International Standard is applicable to products intended for human consumption or the feeding of animals.

The applicability of this International Standard to the examination of certain fermented food and animal feeding stuffs is limited. For the examination of fermented food and animal feeding stuffs, other media and/or incubation conditions might be more appropriate.

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 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

3 Term and definition

For the purposes of this document, the following term and definition applies.

3.1
microorganism
bacteria, yeast and mould-forming countable colony, produced under the conditions specified in this International Standard

4 Principle

4.1 Two poured plates are prepared using a specified culture medium and a specified quantity of the test sample, if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products.

Other pairs of poured plates are prepared, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

4.2 The plates are aerobically incubated at 30 °C for 72 h.

4.3 The number of microorganisms per millilitre or per gram of sample is calculated from the number of colonies obtained on selected plates (see Clause 10).

5 Culture media and diluents

For current laboratory practice, see ISO 7218 and ISO/TS 11133-1.

5.1 Diluents

See the relevant part of ISO 6887.

5.2 Plate count agar (PCA)

5.2.1 Composition

Enzymatic digestion of casein	5,0 g
Yeast extract	2,5 g
Glucose, anhydrous ($C_6H_{12}O_6$)	1,0 g
Agar[1)]	9 g to 18 g
Water	1 000 ml

When dairy products are examined, add 1,0 g of skimmed milk powder per litre of the culture medium. The skimmed milk powder shall be free from inhibitory substances.

5.2.2 Preparation

5.2.2.1 Preparation from commercial dehydrated complete medium

Follow the manufacturer's instructions and add, if necessary, the skimmed milk powder (see 5.2.1).

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

5.2.2.2 Preparation from dehydrated basic components

Dissolve and disperse in the water, in the following order: the yeast extract, the enzymatic digestion of casein, the glucose and, if necessary, the skimmed milk powder. Heating the water will assist this procedure.

Add the agar and heat to boiling, stirring frequently until the agar is completely dissolved.

1) Depending on the gel strength of the agar.

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

5.2.2.3 Distribution, sterilization and storage

Dispense the medium into test tubes (6.8), in quantities of 12 ml to 15 ml per tube, or into flasks or bottles (6.8) of capacity not greater than 500 ml.

Sterilize in an autoclave at 121 °C for 15 min.

If the medium is to be used immediately, cool it to 44 °C to 47 °C in a water bath (6.5) before use. If not, store it in the dark at a temperature of 3 °C ± 2 °C for no longer than 3 months, under conditions which do not allow any change in its composition and properties.

Before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium, then cool it to 44 °C to 47 °C in a water bath (6.5) before use.

In order to check the temperature of the agar, it is recommended to place a thermometer into a portion of 15 g/l agar control solution in a separate container identical to that used for the medium. The temperature control solution should be exposed to the same heating and cooling operations as the medium itself.

5.2.3 Performance testing for the quality assurance of the culture medium

To test the performance of the medium, see ISO/TS 11133-1.

5.3 Overlay medium (if necessary; see 9.2.7)

5.3.1 Composition

Agar[1)]	12 g to 18 g
Water	1 000 ml

5.3.2 Preparation

Add the agar to the water and heat to boiling, stirring frequently until the agar is completely dissolved, or steam for about 30 min.

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

5.3.3 Distribution, sterilization and storage

Dispense the medium into test tubes (6.8) in quantities of 4 ml per tube, or into flasks or bottles (6.8) of appropriate capacity.

Sterilize in an autoclave at 121 °C for 15 min.

If the medium is to be used immediately, cool it to 44 °C to 47 °C in a water bath (6.5) before use. If not, store it in the dark at a temperature of 3 °C ± 2 °C for no longer than 3 months, under conditions which do not allow any change in its composition and properties.

Before beginning the microbiological examination, in order to avoid any delay when pouring the medium, completely melt the medium, then cool it to 44 °C to 47 °C in a water bath (6.5) before use.

6 Apparatus and glassware

Disposable glassware is an acceptable alternative to re-usable glassware if it has suitable specifications.

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

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

See ISO 7218.

6.2 Incubator, capable of operating at 30°C ± 1°C.

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

6.4 Pipettes, of nominal capacity 1 ml.

6.5 Water bath, capable of operating at 44 °C to 47 °C.

6.6 Colony-counting equipment, for example, consisting of an illuminated base with a dark background, fitted with magnifying lens of suitable magnification of about 1,5 × may be used and a mechanical or electronic digital counter.

6.7 pH-meter, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.

6.8 Test tubes, flasks or **bottles,** of appropriate capacity and not greater than 500 ml.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. See the specific International Standard dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

8 Preparation of test sample

Prepare the test sample in accordance with the relevant part of ISO 6887, or ISO 8261, and the specified standard dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 Test portion, initial suspension and dilutions

See the relevant part of ISO 6887 and the specific International Standard dealing with the product concerned.

9.2 Inoculation and incubation

9.2.1 Take two sterile Petri dishes (6.3). Transfer to each dish, by means of a sterile pipette (6.4) 1 ml of the test sample, if liquid, or 1 ml of the initial suspension in the case of other products (10^{-1} dilution).

9.2.2 Take two other sterile Petri dishes (6.3). Transfer to each dish, by means of another sterile pipette (6.4), 1 ml of the 10^{-1} dilution (liquid product) or 1 ml of the 10^{-2} dilution (other products).

9.2.3 If necessary, repeat the procedure with the further dilutions, using a new sterile pipette for each decimal dilution.

9.2.4 If appropriate and possible, select only the critical dilutions steps (at least two consecutive decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 15 and 300 colonies per plate.

9.2.5 Pour about 12 ml to 15 ml of the plate count agar (5.2) at 44 °C to 47 °C into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium (5.2) is poured into the dishes shall not exceed 45 min.

9.2.6 Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface.

9.2.7 After complete solidification, and only in the case where it is suspected that the product under examination contains microorganisms whose colonies will overgrow the surface of the medium, pour about 4 ml of the overlay medium (5.3) at 44 °C to 47 °C onto the surface of the inoculated medium. Allow to solidify as described above.

9.2.8 Invert the prepared dishes and place them in the incubator (6.2) at 30 °C ± 1 °C for 72 h ± 3 h. Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

9.3 Counting of colonies

9.3.1 After the specified incubation period (9.2.8), count the colonies on the plates (10.1), using the colony-counting equipment (6.6) if necessary. Examine the dishes under subdued light. It is important that pinpoint colonies should be included in the count, but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, in order to distinguish colonies from foreign matter.

9.3.2 Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the dish and calculate the corresponding number of the entire dish. If more than one-quarter is overgrown by spreading colonies, discard the count.

10 Expression of results

10.1 Method of calculation

See Amendment 1 to ISO 7218:1996.

10.2 Precision

10.2.1 General

The precision data were evaluated for dishes containing more than 15 and fewer than 300 colonies. The precision data depend on the flora association and the sample matrix. The data presented are derived from collaborative studies (see references [1], [2] and [3]) and are valid for raw and pasteurized milk. They may be used as estimates when colony counts in other products are determined.

10.2.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than the repeatability limit, $r = 0{,}25$, in $\log_{10}$ microorganisms per millilitre (corresponding to 1,8 on the normal scale in microorganisms per millilitre).

NOTE This repeatability limit was derived from collaborative studies for raw and pasteurized milk (see references [1], [2] and [3]) and may be used for such products.

10.2.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit, $R = 0{,}45$, in $\log_{10}$ microorganisms per millilitre (corresponding to 2,8 on the normal scale in microorganisms per millilitre).

NOTE This reproducibility limit was derived from collaborative studies for raw and pasteurized milk (see references [1], [2] and [3]) and may be used for such products.

10.3 Interpretation of test results

In the following examples, the average precision data, a probability level of 95 % and the analysis of one sample are considered. It should be noted that, under practical conditions, the average of several samples is often used. The figures are indicated in microorganisms per millilitre.

a) **Repeatability conditions**

First result: $10^5 = 100\ 000$

The difference between the first and the second result should not be greater than 0,25 $\log_{10}$ units.

Second result: $\log 10^{4,75} = 56\ 000$ or

$\log 10^{5,25} = 178\ 000$

The difference between the first and the second result is acceptable if the second result is not lower than 56 000 or not higher than 178 000.

b) **Reproducibility conditions**

Results obtained in the first laboratory (average of duplicate determination): $10^5 = 100\ 000$

The difference between the first and the second result obtained in the second laboratory should not be greater than 0,45 $\log_{10}$ units:

Second results: $\log 10^{4,55} = 36\ 000$, or

$\log 10^{5,45} = 280\ 000$

The difference between the results obtained by the first and the second laboratory is acceptable, if the second laboratory obtains a result which is not lower than 36 000 and not higher that 280 000.

Annex A shows the calculation and use of the critical difference (CD) to interpret results.

10.4 Confidence limits

See ISO 7218.

11 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the results;

e) the test results obtained.

Annex A
(informative)

Use of the Critical Difference (CD) for the interpretation of results

In the following examples, the average precision data, a probability level of 95 % and the analysis of one sample are considered. It should be noted that, under practical conditions, the average of several samples is often used. The figures are indicated in microorganisms per millilitre.

a) **Reproducibility conditions**

Results obtained in the first laboratory (average of duplicate determination): $10^5 = 100\ 000$

The difference between this result and a result obtained by a second laboratory (average of n determinations; $n = 2$ in this example) is acceptable if it does not exceed the critical difference (CD), in $\log_{10}$ units:

$$\mathrm{CD} = \sqrt{R^2 - r^2\left(1 - \frac{1}{n}\right)} = \sqrt{R - \frac{r^2}{2}} = \sqrt{0{,}45 - \frac{0{,}25^2}{2}} = 0{,}41$$

where

r is the repeatability limit;

R is the reproducibility limit.

The difference between the results obtained by the first and the second laboratory is acceptable if the second laboratory obtains a result which is not lower than $10^{4{,}59} = 39\ 000$ or not higher that $10^{5{,}41} = 257\ 000$.

b) **Comparison with a limit** (one-sided test)

Limit: $10^5 = 100\ 000$

The difference between the limit and the laboratory result (average of n determinations; $n = 2$ in this example) has to be compared to the critical difference limit (CDL):

$$\mathrm{CDL} = 0{,}84\sqrt{2} \times \sqrt{R^2 - r^2\left(1 - \frac{1}{n}\right)} = 0{,}84\sqrt{2} \times \sqrt{R^2 - \frac{r^2}{2}} = 0{,}24$$

Test results up to $10^{5{,}24} = 174\ 000$ do not indicate non-compliance with the limit.

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