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BS ISO 15213:2003

Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions

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Summary of pages

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INTERNATIONAL STANDARD

ISO 15213

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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions

Microbiologie des aliments — Méthode horizontale pour le dénombrement des bactéries sulfito-réductrices se développant en conditions anaérobies

Reference number
ISO 15213:2003(E)

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Foreword

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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 specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt will 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 this method in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain group of products International Standards and/or national standards may already exist that do not comply with this horizontal method. 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.

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Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions

1 Scope

This International Standard specifies a horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. It is applicable to

— products intended for human consumption and the feeding of animals, and

— environmental samples in the area of food production and food handling.

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 6887-1:1999, *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 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

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

ISO 7218:1996/Amd.1:2001, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations — Amendment 1*

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 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
sulfite-reducing bacteria growing under anaerobic conditions
bacteria forming countable typical colonies under the conditions specified in this International Standard

4 Principle

4.1 Two agar plates (or tubes) are prepared, using iron sulfite medium, and using 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.

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Two other agar plates (or tubes) are prepared, under the same conditions, using decimal dilutions of the test sample or the initial suspension.

4.2 The plates (or tubes) are incubated under anaerobic conditions at 37 °C ± 1 °C for 24 h to 48 h (final reading after 48 h), or possibly at 50 °C if thermophilic bacteria are suspected. Typical black-coloured colonies are counted. The black colour of the colonies and the surrounding zone is due to the formation of iron(II) sulfide as a result of the reaction between sulfide ions and trivalent iron [Fe(III)] present in the medium.

4.3 The number of sulfite-reducing bacteria per millilitre or per gram of sample is calculated from the number of colonies obtained on the plates (or tubes).

5 Culture medium and diluent

For current practices, see ISO 7218.

5.1 Plate count medium: Iron sulfite agar

5.1.1 Composition

Enzymatic digest of casein	15 g
Pancreatic digest of soya	5 g
Yeast extract	5 g
Disodium disulfite ($Na_2S_2O_5$)	1 g
Iron(III) ammonium citrate	1 g
Agar	9 g to 18 g[a]
Water	1 000 ml

[a] Depending on the gel strength of the agar.

5.1.2 Preparation

Dissolve the ingredients in the water by heating.

If necessary, adjust the pH so that after sterilization it is 7,6 ± 0,2 at 25 °C.

Pour 250 ml portions of the medium into 500 ml flasks.

If the enumeration is performed by use of tubes (6.5), pour 20 ml or 25 ml of medium into the tubes. Sterilize for 15 min in an autoclave set at 121 °C.

Just before use, de-aerate the medium.

5.2 Saline peptone diluent

See ISO 6887-1:1999, 5.2.1.

6 Apparatus and glassware

Usual microbiological equipment (see ISO 7218) and, in particular, the following.

6.1 Homogenization equipment, for samples of solid food (see ISO 7218).

6.2 Water bath, capable of being maintained at between 44 °C and 47 °C.

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6.3 Anaerobic jars, with equipment for generating an anaerobic atmosphere, and including a system to check the anaerobic conditions.

6.4 Incubator, capable of being maintained at 37 °C ± 1 °C and, if necessary, at 50 °C ± 1 °C.

6.5 Test tubes, of dimensions 16 mm × 160 mm, and **flasks** or **bottles** of capacity 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. If there is no specific International Standard dealing with sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

8 Preparation of test sample

Prepare the test samples in accordance with ISO 6887-1, ISO 8261 or the specific International Standard appropriate to the product concerned. If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 General

A diagram of procedure is given in Annex A.

9.2 Test portion, initial suspension and dilutions

See ISO 6887-1, ISO 8261 or any specific International Standard appropriate to the concerned product.

Heat treatment of the initial suspension may be necessary to eliminate vegetative forms of spore-forming bacteria and/or non-spore-forming bacteria. Temperatures and heating times vary according to the actual need, from combinations producing a definite pasteurization effect at a moderate heat activation effect (e.g. 75 °C for 20 min), to boiling for several minutes. In this case, results could be given as number of spores of sulfite-reducing bacteria growing under anaerobic conditions.

9.3 Inoculation

Take two sterile Petri dishes. Using a sterile pipette, transfer to each dish 1 ml of test sample if the product is liquid, or 1 ml of the initial suspension in the case of other products.

Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample if the product is liquid, or 1 ml of the first decimal dilution of the initial suspension (10^{-2}) in the case of other products.

Repeat the described procedure with the further dilutions, using a fresh sterile pipette for each dilution.

Pour into each Petri dish approximately 15 min of iron sulfite agar (5.1) which has been cooled to 44 °C to 47 °C in the water bath (6.2). The time elapsing between inoculation of the Petri dishes and addition of the agar should not exceed 15 min. Carefully mix the inoculum with the medium by horizontal movements and allow the medium to solidify.

After the medium has solidified, pour 5 ml to 10 ml of the same medium into the dish as an overlay.

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If tubes are used, inoculate a 1 ml volume from each dilution into each of two tubes of medium kept at 44 °C to 47 °C. Mix gently without forming bubbles, and leave the medium to solidify in a cold water bath (6.2).

After the medium has solidified, pour 2 ml to 3 ml of the same medium into each tube as an overlay.

9.4 Incubation

After solidification, incubate the Petri dishes in anaerobic jars (6.3) at 37 °C ± 1 °C for 24 h to 48 h.

If thermophilic bacteria are suspected, prepare a second set of Petri dishes (see 9.3). Incubate this set at 50 °C ± 1 °C.

In the case of tubes, incubation in anaerobic jars is not necessary.

9.5 Counting of the colonies

Read the results after 24 h and 48 h, depending on the degree of black colour and the growth rate of the microoganisms. Black colonies, possibly surrounded by a black zone, are counted as sulfite-reducing bacteria.

NOTE 1 Diffuse, unspecific blackening of the medium may occur, especially when inoculation is performed in agar tubes instead of Petri dishes. The growth of anaerobic bacteria, which only produce hydrogen (not H_2S), may also reduce the sulfite present and lead to a general blackening of the medium.

Count colonies of sulfite-reducing bacteria in each dish containing less than 150 typical colonies and less than 300 total colonies.

When the number of colonies is high, some tubes may be unreadable. In this case, only tubes where the colonies are clearly separate should be considered for counting.

NOTE 2 This International Standard may be used to enumerate only *Clostridium*. After obtaining characteristic colonies, pick five of them from each dish, and confirm the genus *Clostridium* with confirmation tests (e.g. respiratory tests, spore-forming tests).

10 Expression of results and confidence limits

See Amendment 1 to ISO 7218:1996.

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, including the temperature of incubation, use of tubes, and any thermal treatment to destroy vegetative bacteria;

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 test results;

e) the test results obtained.

The test report shall also state if further tests are to be carried out by a reference laboratory, or, if already carried out, what the results were.

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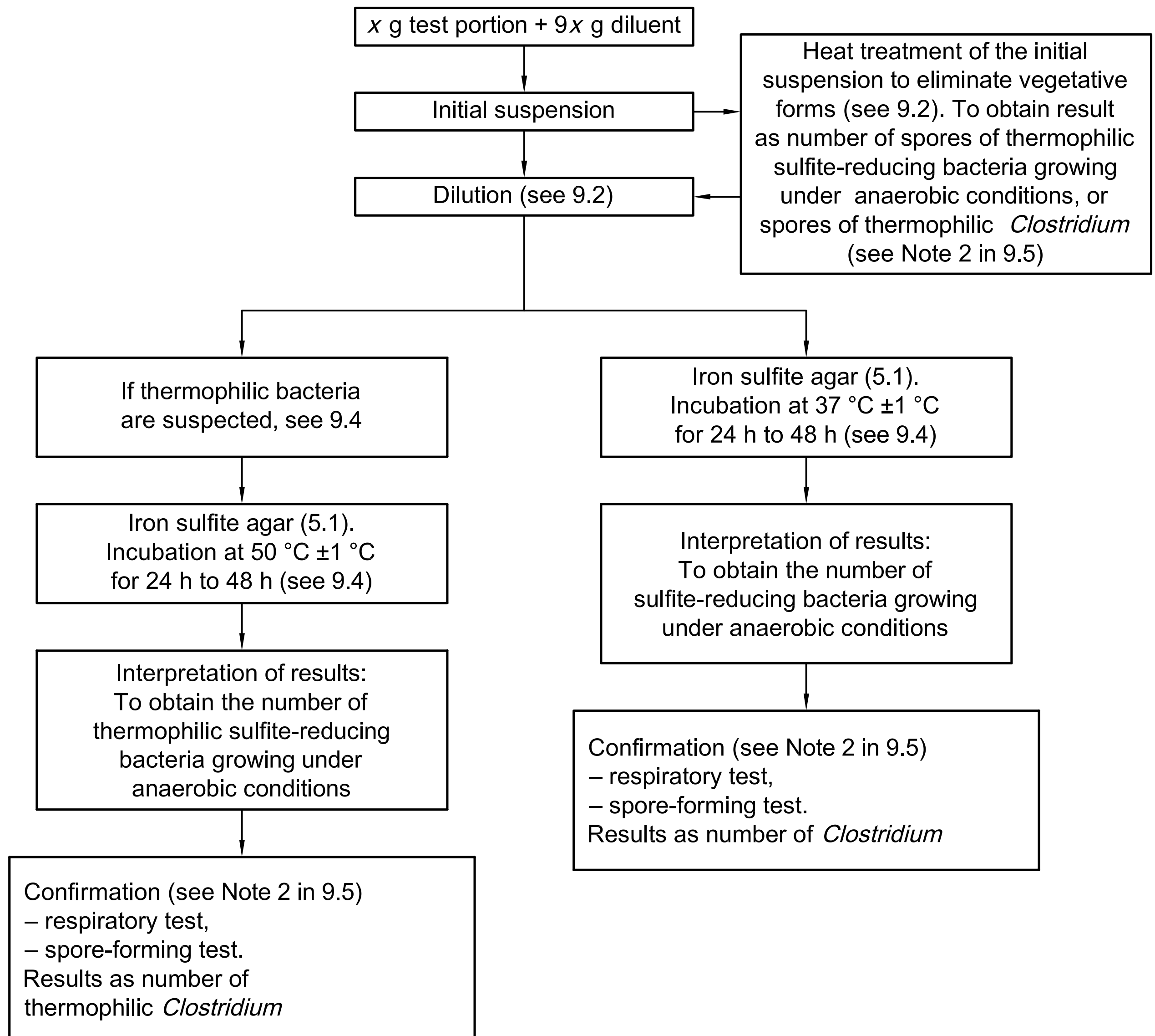
Annex A
(normative)

Diagram of procedure



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[1] NMKL No. 95:1997, *Sulfite-reducing* Clostridia — *Determination in food*[1)]

1) This revised NMKL method has been elaborated by Reidar Skjelkvåle. Oslo City Food Control Authority, Vestbyvn. 13, N-0976 Oslo, Norway.

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