INTERNATIONAL STANDARD

ISO 13720

Second edition
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Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.

Viande et produits à base de viande — Dénombrement des Pseudomonas *spp. présomptifs*

Reference number
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Foreword

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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 13720 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 13720:1995), which has been technically revised.

Meat and meat products — Enumeration of presumptive *Pseudomonas* spp.

1 Scope

This International Standard specifies a method for the enumeration of presumptive *Pseudomonas* spp. present in meat and meat products, including poultry.

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 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

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*

ISO/TS 11133-2, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

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

3.1
presumptive *Pseudomonas* spp.
bacteria which at 25 °C form colonies in cephalothin-sodium fusidate-cetrimide (CFC) agar and which show a positive oxidase reaction when tested according to the method described in this International Standard

4 Principle

An initial suspension and decimal dilutions are prepared from the test sample.

The solid selective medium, CFC agar, is inoculated with a specified quantity of the initial suspension of the product.

Other plates are prepared under the same conditions, using decimal dilutions of the initial suspension.

The plates are incubated at 25 °C for 44 h ± 4 h.

Colonies of presumptive *Pseudomonas* spp. are confirmed by the oxidase test (positive).

The number of presumptive *Pseudomonas* spp. per millilitre, or per gram, of the test sample is calculated from the number of confirmed colonies per plate.

5 Diluent, culture medium and reagent

5.1 General

For current laboratory practice, see ISO 7218; for preparation and testing of media, see ISO/TS 11133-1 and ISO/TS 11133-2.

5.2 Diluent

See ISO 6887-1 and ISO 6887-2.

5.3 Cephalothin-sodium fusidate-cetrimide agar (see Reference [3])

5.3.1 Basic medium

5.3.1.1 Composition

Enzymatic digest of gelatin	16,0 g
Enzymatic digest of casein	10,0 g
Potassium sulfate (K_2SO_4)	10,0 g
Magnesium chloride ($MgCl_2$)	1,4 g
Agar[a]	12,0 g to 18,0 g
Water	1 000 ml

[a] The mass used depends on the gel strength of the agar.

5.3.1.2 Preparation

Dissolve the basic components or the dehydrated basic medium in the water, by bringing to the boil.

Adjust the pH (6.4), if necessary, so that after sterilization it is 7,2 ± 0,2 at 25 °C.

Dispense the basic medium into flasks or bottles of suitable capacity (6.6).

Sterilize for 15 min in the autoclave (6.1) at 121 °C.

5.3.2 Inhibitor solutions

Do not keep solutions for more than 7 days at 5 °C ± 3 °C.

5.3.2.1 Cephalothin solution

5.3.2.1.1 Composition

Cephalothin sodium salt	0,1 g
Water	100 ml

5.3.2.1.2 Preparation

Dissolve the cephalothin in the water and sterilize the solution by filtration.

5.3.2.2 Sodium fusidate solution

5.3.2.2.1 Composition

Sodium fusidate	0,1 g
Water	100 ml

5.3.2.2.2 Preparation

Dissolve the sodium fusidate in the water and sterilize the solution by filtration.

5.3.2.3 Cetrimide solution

5.3.2.3.1 Composition

Cetrimide[a]	0,1 g
Water	100 ml
[a] Mixture consisting chiefly of tetradecyltrimethylammonium bromide together with smaller amounts of dodecyltrimethylammonium bromide and cetrimonium (hexadecyltrimethylammonium) bromide.	

5.3.2.3.2 Preparation

Dissolve the cetrimide in the water and sterilize the solution by filtration.

5.3.3 Complete medium

5.3.3.1 Composition

	Volume ml	Final concentration µg/ml
Basic medium (5.3.1)	100	—
Cephalothin solution (5.3.2.1)	5	50
Sodium fusidate solution (5.3.2.2)	1	10
Cetrimide solution (5.3.2.3)	1	10

5.3.3.2 Preparation

Add the inhibitor solutions to the basic medium, cooled in a water bath (6.3) to 47 °C ± 2 °C, then mix carefully.

5.3.4 Preparation of CFC agar plates

Pour amounts of approximately 15 ml of the complete medium (5.3.3) into sterile Petri dishes (6.8) and allow to solidify.

Immediately before use, the agar plates should be dried in accordance with ISO/TS 11133-1.

If prepared in advance, the undried agar plates shall be kept for not more than 4 weeks at 5 °C ± 3 °C.

5.3.5 Performance testing

For the definition of selectivity and productivity refer to ISO/TS 11133-1. The performance of CFC agar shall be tested according to the methods and criteria described in ISO/TS 11133-2.

5.3.5.1 Productivity

Incubation: At 25 °C ± 1 °C for 44 h ± 4 h

Strain: *Pseudomonas fluorescens* WDCM 00115[1)] or

Pseudomonas fragi WDCM 00116[1)]

Reference medium: Soybean-casein digest agar medium (TSA)

Method of control: Quantitative

Criteria: Productivity ratio $P_R \geqslant 0{,}5$

5.3.5.2 Selectivity

Incubation: At 25 °C ± 1 °C for 44 h ± 4 h

Strain: *Escherichia coli* WDCM 00013[1)]

Method of control: Quantitative

Criteria: Total inhibition

5.4 Reagent for the detection of oxidase

5.4.1 Composition

N,N,N′,N′-Tetramethyl-*p*-phenylenediamine dihydrochloride	1,0 g
Water	100 ml

5.4.2 Preparation

Dissolve the reagent in the water immediately before use.

Commercially available discs or sticks may be used. In this case, follow the manufacturer's recommendations.

6 Apparatus

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

6.1 Oven for dry sterilization or **autoclave for wet sterilization**.

6.2 Incubator, capable of operating at 25 °C ± 1 °C.

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1) Refer to the reference strain catalogue available (viewed 2010-07-19) on http://www.wfcc.nig.ac.jp/WDCM_Reference_Strain_Catalogue for information on culture collection strain numbers and contact details

6.3 **Water bath**, capable of operating at 47 °C ± 2 °C.

6.4 **pH-meter**, capable of measurements to an accuracy of ±0,05 pH units.

6.5 **Loops** made of platinum-iridium alloy or equivalent sterile disposable loops.

6.6 **Test tubes**, **bottles** or **flasks**, of appropriate capacity.

6.7 **Total-delivery pipettes**, sterile, of nominal capacity 1 ml and graduated in 0,1 ml divisions, ISO 835[1] class A, or automatic pipettes, ISO 8655-2[2], with use of sterile tips.

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

6.9 **Spreaders**, made of glass or plastic, e.g. hockey sticks made from a glass rod of approximately 3,5 mm diameter and 200 mm length, bent at right angles about 30 mm from one end and with the cut ends annealed.

7 Sampling

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 interested parties come to an agreement on this subject.

It is important that the laboratory receive a sample which is truly representative and which has not been damaged or changed during transport or storage (see ISO 7218).

8 Preparation of test sample

Prepare the test sample in accordance with ISO 6887-1 and ISO 6887-2 and/or the specific International Standard appropriate to 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

Prepare the initial suspension and dilutions in accordance with ISO 6887-2.

9.2 Inoculation and incubation

9.2.1 In accordance with ISO 7218, one plate per dilution shall be used with at least two successive dilutions. If only one dilution is performed, then two plates shall be used.

9.2.2 Take one CFC agar plate (5.3.4). Transfer, by means of a pipette (6.7), 0,1 ml of the initial suspension on to the plate.

Take another CFC agar plate. Transfer, by means of another sterile pipette, 0,1 ml of the first decimal dilution of the initial suspension on to the plate.

Repeat these operations with subsequent dilutions, using a clean sterile pipette for each decimal dilution.

9.2.3 Spread the liquid over the surface of the agar plate with a sterile spreader (6.9) until the surface is completely dry.