SLOVENSKI STANDARD
SIST EN ISO 13366-3:1998

01-avgust-1998

Mleko - Ugotavljanje števila somatskih celic - 3. del: Fluorooptoelektronska metoda (ISO 13366-3:1997)

Milk - Enumeration of somatic cells - Part 3: Fluoro-opto-electronic method (ISO 13366-3:1997)

Milch - Zählung somatischer Zellen - Teil 3: Fluoreszenzoptoelektronisches Verfahren (ISO 13366-3:1997)

Lait - Dénombrement des cellules somatiques - Partie 3: Méthode fluoro-opto-électronique (ISO 13366-3:1997)

Ta slovenski standard je istoveten z: **EN ISO 13366-3:1997**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology
67.100.10	Mleko in predelani mlečni proizvodi	Milk and processed milk products

SIST EN ISO 13366-3:1998 **en**

EUROPEAN STANDARD

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English version

Milk - Enumeration of somatic cells - Part 3: Fluoro-opto-electronic method (ISO 13366-3:1997)

Lait - Dénombrement des cellules somatiques - Partie 3: Méthode fluoro-opto-électronique (ISO 13366-3:1997)

Milch - Zählung somatischer Zellen - Teil 3: Fluoreszenzoptoelektronisches Verfahren (ISO 13366-3:1997)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

Foreword

The text of the International Standard ISO 13366-3:1997 has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis", the secretariat of which is held by NNI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 1997, and conflicting national standards shall be withdrawn at the latest by December 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the International Standard ISO 13366-3:1997 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 13366-3

First edition
1997-06-15

Milk — Enumeration of somatic cells —

Part 3:
Fluoro-opto-electronic method

Lait — Dénombrement des cellules somatiques —

Partie 3: Méthode fluoro-opto-électronique

Reference number
ISO 13366-3:1997(E)

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 13366 was prepared by Technical Committee ISO/TC 34, *Agricultural food products,* Subcommittee SC 5, *Milk and milk products,* in collaboration with the International Dairy Federation (IDF) and AOAC INTERNATIONAL, and will also be published by these organizations.

ISO 13366 consists of the following parts, under the general title *Milk — Enumeration of somatic cells*:

— *Part 1: Microscopic method*

— *Part 2: Electronic particle counter method*

— *Part 3: Fluoro-opto-electronic method*

Annexes A to D of this part of ISO 13366 are for information only.

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Milk — Enumeration of somatic cells —

Part 3:
Fluoro-opto-electronic method

WARNING — The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 13366 specifies a method for counting somatic cells in both raw and chemically preserved milk, using a fluoro-opto-electronic counting instrument1).

NOTE — Counting of cells in unpreserved samples within the first 24 h after milking could give unreliable results with older instruments (e.g. Fossomatic 90 and 215).

2 Definition

For the purposes of this part of ISO 13366, the following definition applies.

2.1 somatic cells: Those cells that have a minimum intensity of fluorescence due to the staining of DNA in their nuclei.

3 Principle

Mixing of the milk to be examined with a buffer and stain solution. Transference of the mixture in the form of a thin film to a rotating disc, serving as an object plane for a microscope. Each stained cell observed by the microscope produces an electrical pulse that is amplified and recorded. Direct reading of the number of somatic cells in thousands per millilitre.

4 Reagents

WARNING — Ethidium bromide is toxic. The preparation and application of the basic and working solutions shall be carried out in a fume cupboard. Use gloves for protection.

1) The Fossomatic counting instrument (250, 300 or 360) supplied by Foss Electric, Hillerod, Denmark is an example of suitable equipment available commercially. This information is given for the convenience of users of this part of ISO 13366 and does not constitute an endorsement by ISO of the equipment named.

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or deionized water or water of equivalent purity.

4.1 Basic solutions

4.1.1 Dye-buffer solution

4.1.1.1 Composition

Ethidium bromide	2,5 g
Tripotassium citrate	400 g
Citric acid	14,5 g
Deionized water	5 litres
Poly(ethylene glycol) mono-*p*-(1,1,3,3-tetramethylbutyl) phenyl ether[1)]	50 ml

1) For example, Triton X-100 concentrate.

4.1.1.2 Preparation

Dissolve the ethidium bromide in 1 litre of water in a 5 litre container. Stir gently until the ethidium bromide is completely dissolved. The process can be speeded up by heating to between 40 °C and 60 °C. Add the tripotassium citrate and citric acid to the ethidium bromide solution. Add 4 litres of water. Stir gently until the solids are completely dissolved. Add the poly(ethylene glycol) ether concentrate while stirring. Even when stored under light-proof, airtight and cool conditions, the solution shall be kept for no longer than 90 days.

4.1.2 Poly(ethylene glycol) mono-*p*-(1,1,3,3-tetramethylbutyl) phenyl ether solution

4.1.2.1 Composition

Poly(ethylene glycol) mono-*p*-(1,1,3,3-tetramethylbutyl) phenyl ether[1)]	10 ml
Water	1 litre

1) For example, Triton X-100 concentrate.

4.1.2.2 Preparation

Dissolve the poly(ethylene glycol) ether in 1 litre of pre-heated water at approx. 60 °C. Even when stored under airtight and cool conditions, this solution shall be kept for no longer than 25 days.

4.2 Working solution

4.2.1 Dye-buffer working solution

Mix 1 part of the dye-buffer basic solution (4.1.1) with 9 parts of water. (This should be enough for approx. 2 700 samples.) Do not use working solutions older than 7 days.

4.2.2 Rinsing liquid

4.2.2.1 Composition

Poly(ethylene glycol) mono-*p*-(1,1,3,3-tetramethylbutyl) phenyl ether[1)]	10 ml
Ammonia solution, 25 % (*V*/*V*)	25 ml
Water	10 litres
1) For example, Triton X-100 concentrate.	

4.2.2.2 Preparation

Add the poly(ethylene glycol) ether and the ammonia solution to the water.

The composition of the reagents might vary depending on the counting system used. Therefore follow the manufacturer's instructions exactly.

4.3 Preservatives

Boric acid, potassium dichromate, sodium azide or bronopol may be used.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Counting instrument, operating according to the fluorescence optical principle (e.g. Fossomatic). Calibrate in accordance with the manufacturer's instructions. For calibration it is necessary to use milk samples whose cell count has been made by the microscopic method (details are given in ISO 13366-1).

NOTE — Cell count standards are available from the manufacturer.

5.2 Water bath, with circulation, capable of being maintained at a temperature of 40 °C ± 1 °C.

5.3 Sample tubes, with leak-proof seal.

6 Sampling

6.1 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. A recommended sampling method is given in ISO 707 [1].

6.2 If automatic samplers are used, they shall be tested properly.

6.3 Prior to testing or preservation, samples should be stored at a temperature of between 2 °C and 6 °C.

6.4 Preservation, if necessary, shall be carried out as soon as possible after sampling, but in any case within 24 h, by addition of one of the following preservatives.