SLOVENSKI STANDARD
SIST EN ISO 13366-2:1998

01-avgust-1998

Mleko - Ugotavljanje števila somatskih celic - 2. del: Elektronska metoda štetja delcev (ISO 13366-2:1997)

Milk - Enumeration of somatic cells - Part 2: Electronic particle counter method (ISO 13366-2:1997)

Milch - Zählung somatischer Zellen - Teil 2: Elektronisches Partikelzählverfahren (ISO 13366-2:1997)

Lait - Dénombrement des cellules somatiques - Partie 2: Méthode au compteur électronique de particules (ISO 13366-2:1997)

Ta slovenski standard je istoveten z: **EN ISO 13366-2:1997**

ICS:

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

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English version

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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 13366-2:1997 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of an identical text or by endorsement, at the latest by December 1997, and conflicting national standards shall be withdrawn at the latest by December 1997.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 13366-2:1997 was approved by CEN as a European Standard without any modification.

INTERNATIONAL STANDARD

ISO 13366-2

First edition
1997-06-15

Milk — Enumeration of somatic cells

Part 2:
Electronic particle counter method

Lait — Dénombrement des cellules somatiques —

Partie 2: Méthode au compteur électronique de particules

Reference number
ISO 13366-2:1997(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 13366-2 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*

Annex A of this part of ISO 13366 is for information only.

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Milk — Enumeration of somatic cells —

Part 2:
Electronic particle counter 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 the number of somatic cells in both raw and chemically preserved milk, using an electronic particle counter[1)].

NOTE — The user of this method should be aware that due to the counting principle (particle counting) the results are not always comparable with those obtained by the methods of part 1 and part 3 of ISO 13366.

2 Definition

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

2.1 somatic cells: Those cells that are counted by an electronic particle counter, after fixing a lower threshold level and elimination of fat particles overlapping the size range of somatic cells.

3 Principle

Addition of formaldehyde solution (formalin) to the sample to be examined to fix the somatic cells. Dilution by an emulsifying electrolyte mixture and subsequent heating sufficient to break down the fat globules overlapping the size range of the cells. Direct reading of the number of somatic cells in thousands per millilitre.

NOTE — In an electronic particle counter, the milk passes through an aperture located between electrodes. When a particle passes through the aperture, it displaces its own volume of highly conductive liquid by one of lower conductivity. The increased resistance raises the voltage, producing a voltage pulse proportional to the volume of the particle. The number of pulses indicates the number of passing particles. Only pulses above a fixed threshold level are counted.

1) The Coulter Counter, supplied by Coulter Electronics Ltd., Northwell Drive, Luton LV 3 3 RH, Bedfordshire, England, 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.

4 Reagents

WARNING — Formaldehyde is poisonous. The preparation and application of the emulsifier electrolyte mixture shall be carried out in a fume cupboard.

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

4.1 Emulsifier electrolyte mixture

4.1.1 Composition

Ethanol 95 % (*V/V*)	125,0 ml
Poly(ethylene glycol) mono-*p*-(1,1,3,3-tetramethylbutyl) phenyl ether [1]	20,0 ml
Sodium chloride solution, 0,9 g/100 ml	885,0 ml

1) For example, Triton X-100 concentrate.

4.1.2 Preparation

Carefully mix the poly(ethylene glycol) ether and the ethanol. Add the sodium chloride solution. Pass the mixture through an appropriate filter (5.6).

Carry out tests daily in order to determine the number of extraneous particles in the emulsifier electrolyte mixture. This mixture and the plastic and glassware are considered sufficiently clean if the number of particles is below 20 per 0,1 ml of emulsifier electrolyte mixture.

To prevent bacterial growth, 10 ml of formaldehyde solution, 35 % (*m/m*), may be added to the emulsifier electrolyte mixture (4.1).

NOTE — Commercially available emulsifying electrolyte mixture may be used; e.g. Somaton [2] diluent.

4.2 Fixative liquid

4.2.1 Composition

Eosin	0,02 g
Formaldehyde solution, 35 % (*m/m*) [1]	9,40 ml

1) The formaldehyde concentration of commercially available formalin varies between 35 % (*m/m*) and 40 % (*m/m*). This should be taken into account when preparing the fixative liquid.

4.2.2 Preparation

Transfer the eosin and the formaldehyde solution to a 100 ml volumetric flask and mix. Dilute to 100 ml with water and mix again. Filter or centrifuge the liquid in order to remove particles.

NOTE — Eosin is included in the fixative liquid to colour the fixed test samples.

2) Somaton is an example of a suitable product 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 this product.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

Prior to use, all glassware shall be carefully cleaned so as to be as near as possible free from particles.

5.1 Particle counter, electronic particle counter with a capillary tube of 100 µm diameter and counting volumes of 0,1 ml or 0,5 ml (e.g. Coulter Counter models F or FN). Alternatively, an automatic counter (e.g. milk cell counter) may be used with a tube with aperture of 140 µm diameter and a counting volume of 0,3 ml.

CAUTION — When the counter is installed, make sure that any electromagnetic interference is excluded. Both the screen and the time of counting shall be continuously checked.

Calibrate the apparatus before use to determine the relation between the volume of the particles to be counted and the threshold level above which the counts are made. Calibrate in accordance with the manufacturer's instructions by using a standard particle suspension.

Check the calibration by differential counts in some samples with counts between 300 000 cells/ml and 1 000 000 cells/ml. Evidence shall be produced that the modal diameter of the cells is between 5,45 µm and 6,25 µm. Evaluate a threshold value for routine estimation, corresponding to an equivalent diameter of between 4,7 µm and 5,0 µm, depending on the size distribution found. Check each manometer to verify that the counts in 0,1 ml are 1/5 of the count in 0,5 ml (for details, see ISO 13366-3:1997, annex C).

5.2 Water bath, with circulation, capable of being maintained at any temperature between 20 °C and 37 °C.

5.3 Water baths, with circulation, capable of being maintained at temperatures of 55 °C ± 1 °C and 80 °C ± 1 °C.

5.4 Incubator, capable of being maintained at a temperature of 30 °C ± 1 °C.

5.5 Pipetting device, capable of preparing the 1:100 dilution (this is optional, see 8.1.2).

5.6 Filter, resistant to the solvents used, with a pore size of 0,5 µm or less.

5.7 Glass or plastic tubes, for example of 100 mm length and 16 mm diameter, round-bottomed, with a straight brim and appropriate seal.
When plastic tubes are used, tests shall be made to ensure that no loss of somatic cells occurs due to adherence to the surface of the tubes. After the tubes have been rinsed, this shall be repeated with filtered distilled water.

5.8 Pipetting device, capable of dispensing 0,2 ml of fixative liquid.

5.9 Analytical balance, capable of weighing to the nearest 0,01 g.

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 part of ISO 13366. A recommended sampling method is given in ISO 707 [1].

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