# INTERNATIONAL STANDARD

# ISO 13366-2

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## Milk — Enumeration of somatic cells

## Part 2:
Electronic particle counter method

*Lait — Dénombrement des cellules somatiques —*

*Partie 2: Méthode au compteur électronique de particules*

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## Foreword

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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.

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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.

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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

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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]).

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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.

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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

| Component | Quantity |
|---|---|
| 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).

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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).

ISO 13366-2:1997
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NOTE — Commercially available emulsifying electrolyte mixture may be used; e.g. Somaton [2] diluent.

### 4.2 Fixative liquid

#### 4.2.1 Composition

| Component | Quantity |
|---|---|
| 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.

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

ISO 13366-2:1997

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

1056857cc648/iso-13366-2-1997

**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.

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

**6.4** Raw milk samples not to be tested within 6 h after sampling shall be preserved by the addition of boric acid. The final concentration of boric acid shall not exceed 0,6 g per 100 ml of sample. Store such samples at a temperature of between 6 °C and 15 °C for no longer than 24 h.

**6.5** Immediately after sampling, samples should be fixed with formalin (see clause 7). This should be done using sample tubes which already contain the correct amount of the fixation liquid. The tubes shall be kept sealed to prevent evaporation of formalin.

## 7 Preparation of test sample

After thorough mixing, take 10 ml of the sample. Add 0,2 ml of the fixative liquid (4.2) dispensed by the pipetting device (5.8). Mix to fix the somatic cells.

Normally fixation of the cells is done by adding formaldehyde to milk in the proportion of about 1:1 500, that is, by the addition of 0,2 ml of fixative liquid to 10 ml of test sample. Higher formaldehyde concentrations may be used [for example in the proportion of about 1:500 by raising the amount of formaldehyde solution, 35 % (*m*/*m*), in the fixative liquid to 30,0 ml] but special precautions should then be taken to avoid falsely elevated counts [for example, by heating milk samples to 55 °C in the water bath (5.3) for 50 min].

Keep the test samples at 30 °C in the incubator (5.4) for 15 h to 18 h, or between 18 °C and 25 °C for 22 h to 26 h.

Fixed samples should not be stored for more than 48 h at 6 °C to 15 °C, in order to ensure that the precision of counts remains within the limits specified (for details, see ISO 13366-3:1997, annex B).

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ISO 13366-2:1997
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## 8 Procedure

### 8.1 Test portion

**8.1.1** Warm refrigerated fixed test samples in the water bath (5.2) to a temperature of between 20 °C and 37 °C. After thorough mixing, transfer 0,1 ml of the test sample to each tube (5.7) and dilute with the emulsifier electrolyte mixture (4.1) to give 10 ml.

**8.1.2** This dilution may be carried out either manually or by using the pipetting device (5.5). Variation should not exceed ± 1,5 %. Dilution accuracy should be checked regularly by weighing, carrying out at least 20 separate determinations, and using a well-mixed test sample.

### 8.2 Dispersion of fat particles

Heat the test portion (8.1) in the water bath (5.3) to 80 °C for 10 min. Check the temperature of the water bath with a control blank to ensure that the test portion reaches and maintains the correct temperature. Take care that the water bath contains sufficient water to keep the level of the liquid in the tubes below the water level.

Remove the test portion from the water bath and cool it to a temperature of between 15 °C and 25 °C.

NOTE — Slight opalescence of the test portions after heat treatment is due to hardening of the casein micelles by formalin. These micelles have a diameter of less than 1 µm and do not affect the counting.

### 8.3 Determination

Count the cells in the test portion (8.2) within 1 h of cooling it. Thoroughly mix the test portion immediately before counting so as to obtain as homogeneous a distribution of the cells as possible. Transfer the test portion to a measuring vessel. Take care that no air bubbles are produced and that sedimented cells are not retained in the tubes.

Then perform counting by the electronic particle counter (5.1). During counting, the outer electrode of the aperture tube shall be below the surface of the liquid.

During counting, the pulse monitor should be checked in order to detect possible interference. In addition, the time for counting each test portion should be within the tolerance.

## 9 Expression of results

With a measuring volume of 0,1 ml and a dilution of 0,1 ml of milk in 10 ml of emulsifier electrolyte mixture (4.1), the number of somatic cells is given, as a direct reading, in thousands per millilitre of milk.

For a discussion of the use of cell-count standard samples, see ISO 13366-3:1997, annex C.

## 10 Precision

Annex B of ISO 13366-3:1997 gives recommendations for procedures for quality control and interlaboratory testing.

## 11 Test report

The test report shall specify:

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— the method in accordance with which sampling was carried out, if known;

— the method used;

— the test result(s) obtained; and

— if the repeatability has been checked, the final quoted result obtained.

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It shall also mention all operating details not specified in this part of ISO 13366, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

The test report shall include all information necessary for the complete identification of the sample.

# Annex A
(informative)

# Bibliography

[1] ISO 707:1997, *Milk and milk products — Guidance on sampling.*

[2] ISO 13366-1:1997, *Milk — Enumeration of somatic cells — Part 1: Microscopic method.*

[3] ISO 13366-3:1997, *Milk — Enumeration of somatic cells — Part 3: Fluoro-opto-electronic method.*

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