INTERNATIONAL STANDARD

ISO 13366-1

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Milk — Enumeration of somatic cells —

Part 1:
Microscopic method

Lait — Dénombrement des cellules somatiques —

Partie 1: Méthode au microscope

Reference number
ISO 13366-1:1997(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 13366-1 was prepared by Technical Committee ISO/TC 34, *Agricultural food 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 1:
Microscopic 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. The method is suitable for preparing standard test samples and for calibrating mechanized and automatic cell-counting procedures.

2 Definition

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

2.1 somatic cells: Those cells with nuclei, that is, all leucocytes and epithelial cells.

3 Principle

Spreading of a test portion of the milk to be examined over a slide to form a film. Drying and staining of the film and subsequent counting of the stained cells using a microscope. Multiplication of the number of cells counted in a defined area by a working factor to give the number of cells per millilitre.

4 Reagents

WARNING — Tetrachloroethane is poisonous. Ethidium bromide is toxic. The preparation and application of the dye solution shall be carried out in a fume cupboard. Use gloves for protection.

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

4.1 Dye solution

4.1.1 Composition

Ethanol 95 % (*V*/*V*)	54,0 ml
Tetrachloroethane	40,0 ml
Methylene blue	0,6 g
Acetic acid, glacial	6,0 ml

NOTE — As an alternative, tetrachloroethane may be replaced by the same amount of trichloroethane. Instead of methylene blue, ethidium bromide can be used (see ISO 13366-3).

4.1.2 Preparation

Mix the ethanol and tetrachloroethane in a bottle. Heat in the water bath (5.1) set at 65 °C. Add the methylene blue and mix carefully. Cool in a refrigerator to 4 °C and then add the glacial acetic acid. Pass the solution through an appropriate filter (5.3) into an airtight bottle and store it as such. If necessary, filter again before use.

5 Apparatus

Usual laboratory equipment and, in particular, the following.

5.1 Water bath, capable of being maintained at a temperature of 65 °C ± 5 °C.

5.2 Water bath, capable of being maintained at a temperature of 35 °C ± 5 °C.

5.3 Filter, resistant to the solvents used, with a pore size of between 10 µm and 12 µm or less.

5.4 Microscope, with magnification of between × 500 and × 1 000.

If ethidium bromide is used, the microscope shall have fluorescence equipment.

5.5 Microsyringe, of capacity 0,01 ml, with maximum tolerance of 2 %.

5.6 Slides, marked with the outline of a shape of a film of dimensions 20 mm × 5 mm, or a standard slide and a template of dimensions 20 mm × 5 mm.

5.7 Hot-plate, capable of being maintained at a temperature of 40 °C ± 10 °C.

5.8 Fan, hairdryer type.

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 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 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 2 °C and 6 °C for no longer than 24 h.

7 Preparation of test sample

Heat the test sample in the water bath (5.2) set at a temperature of 35 °C. Mix the test sample carefully and cool to the temperature at which the microsyringe has been calibrated, for example to 20 °C.

8 Procedure

Prepare and count from each test sample at least two films. Clean the slides (5.6), for example with ethanol. Dry them with dust-free paper, flame and cool.

8.1 Test portion and preparation of film

Use the microsyringe (5.5) to take 0,01 ml of the prepared test sample (clause 7). Carefully clean the outside of the syringe which has been in contact with the sample. Place the test portion on a clean slide with the outline of the shape (20 mm × 5 mm) (5.6). Then fill in this area as evenly as possible with the test portion. Dry the film on a level hot-plate (5.7) until completely dry. Better results can be obtained by drying the films at ambient temperature for several hours.

Dip the dried film on the slide in the dye solution (4.1) for 10 min. Complete drying with the fan (5.8) if required. Then dip the film in tap water until all surplus dye is washed away. Dry again and store with protection against dust.

8.2 Determination

Using the microscope (5.4), count the cell nuclei in the film (at least 400). These are clearly recognizable and at least half should be visible in the microscope field. Count nuclei in vertical strips in the middle third of the film. Avoid counting strips selected exclusively from the peripheral areas of the film.

Check at least once a month the proper preparation of the films, and hence the reliability of the results, by counting different parts of the film.

9 Calculation and expression of results

9.1 The length of the strips to be counted is 5 mm each. The width of a strip corresponds to the diameter of the microscope field. With a test portion of 0,01 ml of sample, calculate the working factor, w_f, using the following equation:

$$w_f = \frac{20 \times 100}{d \times b}$$

where

d is the numerical value of the diameter, in millimetres, of the microscope field;

b is the number of strips counted completely.

9.2 The number of somatic cells is multiplied by the working factor, w_f, to give the number of cells per millilitre of sample.

10 Precision

10.1 Repeatabililty and reproducibility

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

10.2 Minimum number of cells to be counted

Microscopic counting of somatic cells may also be used for the calibration of automatic and mechanized counting procedures. Therefore, the coefficient of variation of counts on identical samples will not be higher than that of electronic instruments. The coefficient of variation of a milk sample containing 400 000 to 600 000 cells per millilitre, with approximately 80 % neutrophils, shall not exceed 5 %.

To meet this requirement, the number of somatic cells to be counted in each sample shall be at least 400. The Poisson distribution presupposes that

$$M = V = s_d^2$$

where

M is the mean value;

V is the variance;

s_d is the standard deviation.

The coefficient of variation (CV) is

$$CV = \frac{s_d \times 100}{M} \% \text{ or}$$

$$CV = \frac{100}{s_d} \% \text{ or}$$

$$CV = \frac{100}{\sqrt{M}} \%$$

where M is the mean value which, in the case of counting the number of somatic cells, is the number of particles (cells) counted.

11 Test report

The test report shall specify:

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

— the method used;

— the working factor of microscopy;

— the test result(s) obtained; and

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

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 13366-2:1997, *Milk — Enumeration of somatic cells — Part 2: Electronic particle counter method.*

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

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