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Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination

Lait et produits laitiers — Lignes directrices générales pour la préparation des échantillons pour essai, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique

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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.

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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.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 8261 | IDF 122 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This edition cancels and replaces the first edition (ISO 8261:1989), which has been technically revised.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Preparations of samples and dilutions for microbiological examinations*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Mr L.J.M. Maturin (US).

This edition cancels and replaces the third edition (IDF 122C:1996).

Introduction

This International Standard is mainly based on ISO 6887-1. The necessary adaptations to microbiological laboratory practice in the dairy industry and instructions specific to dairy products, especially in relation to sample preparation, have been introduced.

The question of which diluent or diluents to specify has been the subject of discussion for some time. In this International Standard the peptone/saline solution, as well as the buffered peptone water solution as used in ISO 6887-1, is specified. Three other diluents which are commonly used in dairy microbiological laboratories are also specified for general use. Furthermore, six diluents are specified for special purposes in dairy microbiological laboratories.

Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination

1 Scope

This International Standard describes general guidelines for the preparation of test samples, initial suspensions and decimal dilutions for the microbiological examination of milk and milk products, including milk-based infant foods.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

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 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

3 Terms and definitions

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

3.1
initial suspension
primary dilution
suspension, solution or emulsion obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed, if necessary, using a blender and observing appropriate precautions, with a nine-fold quantity of dilution fluid (diluent), allowing large particles, if present, to settle

NOTE 1 In certain cases and in particular for products giving an initial 1 + 9 suspension which is too viscous or too thick, it may be necessary to add more diluent. On the other hand, a more concentrated primary dilution than 1 + 9 may be required for results of tests to relate to certain specification criteria. These factors should be taken into account for subsequent operations and/or in the expression of results.

NOTE 2 The use of the first dilution is the most appropriate for fitting the requirement of less than 10 microorganisms per gram. If it is desirable for some enumerations in some products to fall below this threshold, it is possible to use less diluent for the suspension. However, inoculation of this suspension may result in an unbalanced inoculum/medium ratio.

NOTE 3 For appropriate precautions, see 8.1.

NOTE 4 For details of diluents, see clause 5.

3.2
further decimal dilutions
suspensions, solutions or emulsions obtained by mixing a specific volume of the primary dilution (3.1) with a nine-fold volume of diluent, and by repeating this operation with every dilution thus prepared, until a decimal dilution series, suitable for the inoculation of culture media, is obtained

NOTE See 8.1.

4 Principle

An initial suspension (3.1) is prepared and, if necessary, further decimal dilutions (3.2) are prepared to reduce the number of microorganisms per unit volume to facilitate microbiological examination.

5 Diluents

5.1 Basic materials

In order to improve the precision of the results, it is recommended that, for the preparation of the diluent, dehydrated basic components or a dehydrated complete preparation be used. The manufacturer's instructions shall be rigorously followed.

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent quality (see ISO 7218).

Any adjustment necessary to the pH of the media shall be made with solutions of sodium hydroxide (NaOH) or hydrochloric acid (HCl) of appropriate molarities to minimize the change in media volume and thus composition; i.e. in general, the lower the volume of medium, the higher the molarity.

5.2 Diluents for general use

5.2.1 Peptone-salt solution

5.2.1.1 Composition

Peptone of enzymatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

5.2.1.2 Preparation

Dissolve the components in the water, by heating slightly on a hot plate (6.13) if necessary. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

5.2.2 Quarter-strength Ringer's solution

5.2.2.1 Composition

Sodium chloride (NaCl)	2,25 g
Potassium chloride (KCl)	0,105 g
Calcium chloride, anhydrous ($CaCl_2$)	0,06 g
Sodium hydrogen carbonate ($NaHCO_3$)	0,05 g
Water	1 000 ml

5.2.2.2 Preparation

Dissolve the salts in the water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 6,9 ± 0,2 at 25 °C.

5.2.3 Peptone solution

5.2.3.1 Composition

Peptone	1,0 g
Water	1 000 ml

5.2.3.2 Preparation

Dissolve the peptone in the water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 7,0 ± 0,2 at 25 °C.

5.2.4 Phosphate buffer solution

5.2.4.1 Composition

Potassium dihydrogen phosphate (KH_2PO_4)	42,5 g
Water	1 000 ml

5.2.4.2 Preparation

Dissolve the salt in 500 ml of water. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 7,2 ± 0,2 at 25 °C. Dilute to 1 000 ml. Store the stock solution under refrigeration.

Add 1 ml of this stock solution (at 20 °C) to 1 000 ml of water for use as diluent.

5.3 Diluents for special purposes

These diluents shall only be used for the preparation of initial suspensions.

5.3.1 Pre-enrichment medium: Buffered peptone water

5.3.1.1 Composition

Peptone of enzymatic digest of animal tissues	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

5.3.1.2 Preparation

Dissolve the components in the water by heating slightly on a hot plate (6.13) if necessary. Adjust the pH with the appropriate solution (5.1) so that after sterilization it is 7,0 ± 0,2 at 25 °C.

This diluent is recommended for cases where tests for detecting *Salmonella* or *Listeria monocytogenes* are also to be undertaken (see ISO 6579).

5.3.2 Sodium citrate solution [for cheese and (roller) dried milk]

5.3.2.1 Composition

Trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	20,0 g
Water	1 000 ml

5.3.2.2 Preparation

Dissolve the salt in water by heating on a hot plate (6.13) at a temperature of between 45 °C to 50 °C. Adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 7,5 ± 0,2 at 25 °C.

5.3.3 Dipotassium hydrogen phosphate solution [for cheese, (roller) dried milk, fermented milk, caseinates, dried acid whey and sour cream]

5.3.3.1 Composition

Dipotassium hydrogen phosphate (K_2HPO_4)	20,0 g
Water	1 000 ml

5.3.3.2 Preparation

Dissolve the salt in the water by heating on a hot plate (6.13) at a temperature of between 45 °C to 50 °C. For acid whey powder, adjust the pH with the appropriate solution (5.1) so that for the primary dilution, after sterilization, it is 8,4 ± 0,2 at 25 °C. For cheese, roller-dried milk, fermented milk, caseinates and sour cream, adjust the pH with the appropriate solution (5.1) so that, after sterilization, it is 7,5 ± 0,2 at 25 °C.

5.3.4 Dipotassium hydrogen phosphate solution with antifoam agent (for acid casein, lactic casein and rennet caseins)

5.3.4.1 Composition

Dipotassium hydrogen phosphate (K_2HPO_4)	20,0 g
Water	1 000 ml

5.3.4.2 Composition of antifoam stock solution

Polyethylene glycol 2000 (BDH)	1 g
Water	1 000 ml

5.3.4.3 Preparation

Dissolve the salt in the water by heating at a temperature of between 45 °C to 50 °C. Add 1 ml of the antifoam stock solution to 1 litre of the K_2HPO_4 solution. Adjust the pH with the appropriate solution (5.1) so that for the primary dilution of both acid and lactic casein, after sterilization, it is 8,4 ± 0,2 at 25 °C, and for rennet casein, after sterilization, it is 7,5 ± 0,2 at 25 °C.

5.3.5 Tripolyphosphate solution (alternative solution for rennet caseins with solubilization problems)

5.3.5.1 Composition

Sodium tripolyphosphate ($Na_3O_{10}P_3$)	20,0 g
Water	1 000 ml

5.3.5.2 Preparation

Dissolve the salt in the water by heating slightly on a hot plate (6.13), if necessary. Dispense the tripolyphosphate solution in bottles in portions of 90 ml and sterilize them in the autoclave (6.1) set at 121 °C for 20 min. The medium may be preserved at a temperature between 0 °C and + 5 °C for a maximum of 1 month.

5.3.6 Diluent for general use with α-amylase solution (for infant food with high starch contents)

Add 12,5 mg of α-amylase (EC 3.2.1.1)[1)] with a specific activity of approximately 400 units[2)] per milligram to 225 ml of the diluent for general use (see 5.2). This diluent is used for 25 g of the test sample. Use analogous amounts of reagents for the preparation of other amount of test samples (e.g. for a 10 g sample, add 5 mg of α-amylase to 90 ml of the diluent for general use).

5.4 Distribution, sterilization and storage of diluent

Dispense the diluent (5.2 or 5.3), preheated to 45 °C if necessary, for the primary dilution into flasks or bottles (6.4). Dispense the diluent for decimal dilutions (5.2) into test tubes (6.5), flasks or bottles (6.4).

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1) The EC number refers to the Enzymatic Classification number as given by the Nomenclature Committee of the International Union of Biochemistry in Enzyme Nomenclature Recommendation (1978), Academic Press, New York, 1979.

2) This unit (often called the International Unit or Standard Unit) is defined as the amount of enzyme which catalyses the transformation of 1 µmol of substrate per minute under standard conditions.

The quantities dispensed shall be such that, after sterilization, each flask or bottle contains 90 ml of diluent or other required quantity and each test tube or bottle contains 9,0 ml of diluent or other required quantity. Stopper the test tubes, flasks or bottles. The uncertainty of measurement of the volumes shall not exceed ± 2 %.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C (a longer period may be necessary for larger volumes). If the diluent is not to be used immediately, store it in the dark at a temperature between 0 °C to 5 °C, for no longer than 1 month, in conditions which do not allow any change in its volume or composition.

If it is necessary to count several groups of microorganisms using different culture media, it may be necessary to distribute all the diluents (or some of them) in quantities greater than 9,0 ml. The size of the test tubes, flasks and bottles (6.4 and 6.5) should be specified accordingly.

6 Apparatus

NOTE Disposable apparatus is an acceptable alternative to reusable glassware, if it has suitable specifications. Reusable glassware should be capable of undergoing repeated sterilization and should be chemically inert.

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

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

See ISO 7218.

NOTE The autoclave can operate either separately or as part of an apparatus for preparing and distributing media.

Pipettes should not be sterilized in an autoclave because moisture will condense on the interior surfaces on cooling and this will affect the accuracy of delivery.

6.2 Blending equipment

See ISO 7218.

The bowls or plastic bags shall have sufficient capacity to allow the sample to be properly mixed with the appropriate amount of diluent. In general, the volume of the container shall be equal to about twice the volume of the test sample plus diluent.

A plastic bag with an integrated filter for retrieving bigger particles may be used.

6.3 Mechanical stirrer, capable of mixing 1 ml or 2 ml of the test sample in the case of liquid products, or the decimal dilutions, in a tube of adequate dimensions with 9 ml or 18 ml of diluent, in order to obtain a homogeneous suspension, and working on the principle of eccentric rotation of the contents of the test tube (e.g. Vortex mixer) (see ISO 7218).

6.4 Flasks or **bottles**, of sufficient capacity to contain and leave adequate head-space for mixing the 90 ml of diluent used for the initial suspension, or multiples of 90 ml.

6.5 Test tubes (flasks or bottles), of sufficient capacity to leave adequate head-space for mixing 10 ml or, if necessary, a multiple of 10 ml of the test sample, if liquid, or of the primary dilution, in other cases, or further decimal dilutions.

6.6 Pipettes, plugged with cotton wool or equipped with a mechanical pipetting device, of nominal capacity 1 ml and having an outlet of diameter 1,75 mm to 3 mm. Use only pipettes with unbroken tips and, when appropriate, having graduations distinctly marked to contrast sharply with the contents.

6.7 Graduated pipettes, plugged with cotton wool or equipped with a mechanical pipetting device, of relatively large capacity, for example 10 ml or 20 ml. Use only pipettes with unbroken tips and, when appropriate, having graduations distinctly marked to contrast sharply with its contents.

6.8 Glass beads, of diameter about 6 mm.

6.9 pH-meter, with temperature compensation, accurate to ± 0,2 pH unit (see ISO 7218).

6.10 Analytical balances, with sufficient weighing capacity and accurate to within 1 % of the net mass being weighed (see ISO 7218).

6.11 Water baths, capable of operating at 30 °C ± 1 °C, 37 °C ± 1 °C, and 45 °C ± 1 °C.

6.12 Spatulas or **glass sticks**

6.13 Hot plate, or other apparatus capable of gentle heating (not gas burners), and capable of operating at the required temperature.

7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

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

If there is no specific International Standard dealing with the product concerned, it is recommended that the parties concerned come to an agreement on the subject.

8 Procedure

8.1 General

During the procedure, the temperature of the test portions, initial suspension and decimal dilutions shall not exceed a temperature of 20 °C, unless otherwise stated.

For some specific investigations (e.g. *Salmonella*), special techniques or precautions may be necessary. In such cases the special techniques are mentioned in the standard for the method in question.

The operations described in 8.2 and 8.3 should not be carried out in direct sunlight.

Normal aseptic precautions should be taken.

8.2 Preparation of test portion and initial suspension

8.2.1 General

Some products will have undergone thermal processing or an acidifying process. For an optimal recovery of stressed microorganisms on selective medium, use reactivation of the primary dilution in order to restore the integrity of these microorganisms. The reactivation consists in leaving the primary dilution at an ambient temperature of 20 °C to 25 °C for 45 min before further dilution or inoculation.

To avoid damaging the microorganisms by sudden changes in temperature, the temperature of the diluent during the operations described below should be approximately the same as that of the test sample unless otherwise specified.

8.2.2 Milk and liquid milk products

Agitate the test sample thoroughly so that the microorganisms are distributed as evenly as possible by rapidly inverting the sample container 25 times. Avoid foaming or allow any foam to disperse. The interval between mixing and removing the test portion shall not exceed 3 min.

Remove 1 ml of the test sample with a sterile pipette (6.6) and add to 9 ml of diluent (5.2) (or 10 ml of test sample to 90 ml of diluent, or 11 ml of test sample to 99 ml of diluent). Shake this primary dilution [e.g. 25 times with a movement of about 300 mm for 7 s manually or, using a mechanical agitator (6.3), for 5 s to 10 s] to obtain a 10^{-1} dilution.

Prepare further dilutions in accordance with 8.3.

8.2.3 Dried milk, dried sweet whey, dried acid whey, dried buttermilk and lactose

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

If the test sample is in the original unopened container and this is too full to permit thorough mixing, transfer it to a larger container, then mix. Open the container, remove the test portion required with a spatula and proceed as indicated below. Immediately close the container again.

Warm a bottle containing 90 ml of diluent in the water bath (6.11) set at 45 °C.

Weigh 10 g of the test sample into a suitable glass vessel (e.g. a beaker) and then add the powder to the dilution bottle containing a suitable diluent (5.2). For dried acid whey, use a special diluent (5.3.3) at pH 8,4 ± 0,2 or, if necessary, for roller-dried milk use a diluent (5.3.2 or 5.3.3) at pH 7,5 ± 0,2.

Alternatively, weigh 10 g of the test sample directly into the bottle with the diluent.

NOTE For better reconstitution and in particular with roller-dried milk, glass beads (6.8) can be helpful. If used they should be added to the bottle before sterilization.

To dissolve the test sample, swirl slowly to wet the powder then shake the bottle 25 times, with a movement of about 300 mm, for about 7 s. A peristaltic-type blender (6.2) may be used as an alternative to shaking.

Replace the bottle in the water bath (6.11) for 5 min, shaking occasionally.

Prepare further dilutions in accordance with 8.3.

8.2.4 Cheese and processed cheese

Weigh 10 g of the test sample in a dish and transfer it to the container of a rotary blender, or a peristaltic-type blender (6.2), or weigh 10 g of the test sample directly into the container.

When a rotary blender or a peristaltic-type blender is used, add 90 ml of diluent (5.3.2 or 5.3.3 at pH 7,5 ± 0,1), preheated to 45 °C.

Blend until the cheese is thoroughly dispersed (1 min to 3 min). If using a rotary blender, operate the equipment for sufficient time to give a total of 15 000 to 20 000 revolutions. Even with the slowest rotary blender this time shall not exceed 2,5 min.

Ideally, ensure that the temperature of the dispersion does not exceed 40 °C and in any case do not allow it to exceed 45 °C. Allow any foam to disperse.

Prepare further dilutions in accordance with 8.3.

8.2.5 Acid casein, lactic casein, rennet casein and caseinate

8.2.5.1 Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

Weigh 10 g of the test sample into a sterile plastic stomacher (see 6.2). Add 90 ml of the appropriate diluent at room temperature, as follows:

— for acid and lactic casein, use as diluent dipotassium hydrogen phosphate solution with antifoam agent (5.3.4) at pH 8,4 ± 0,2;

— for caseinate, use as diluent dipotassium hydrogen phosphate solution (5.3.3) at pH 7,5 ± 0,2; and

— for rennet casein, use as diluent dipotassium hydrogen phosphate solution with antifoam agent (5.3.4) at pH 7,5 ± 0,2.

8.2.5.2 The use of dipotassium hydrogen phosphate solution as diluent (5.3.4) for rennet caseins may cause problems with the solvability of the grains of caseins. These "grains" hamper the enumeration of PCA + L. Therefore, it is recommended to use the following alternative procedure.

Mix well and allow to stand for 15 min at room temperature. Blend for 2 min in the peristaltic blender (6.2) by using two sterile bags for granulated products, if necessary. Allow to stand for 5 min.

Prepare further decimal dilutions in accordance with 8.3.

8.2.5.3 Alternatively, for rennet caseins for which problems are expected with the solvability of grains, use the following procedure.

If necessary, grind dry casein before taking the test specimen. Put approx. 20 g of test sample in a suitable container. Grind it using an apparatus with knives able to turn approx. 20 000 turns per minute, equipped with a device that prevents the sample heating during grinding (e.g. Virtis apparatus).

Weigh 5 g of the thus-prepared test sample in a sterile bottle of capacity 250 ml. Add glass balls for mixing and 95 ml of the sodium tripolyphosphate solution (5.3.5) preheated to 37 °C. Mix by leaving the bottle on a mixing device for 15 min. Then place it in the water bath set at 37 °C for 15 min while mixing from time to time.

Prepare further dilutions in accordance with 8.3.

8.2.6 Butter

Weigh 10 g of the test sample into a sample container. Place the container in the water bath (6.11) set at 45 °C. Keep it in the water bath until the whole test portion has just melted. Add 90 ml of diluent (5.2) warmed to 45 °C and mix. This operation is more easily carried out in a peristaltic-type blender (6.2).

Alternatively use only the aqueous phase for dilution, as follows.

Take a test portion of 50 g [containing a volume/mass fraction of about 16 % (= 8 ml) of water] and add an amount (50 – 50x % of water in the butter) (= 42 ml) of diluent (5.2.4) prewarmed on the water bath (6.11) to 45 °C. Place a container in the water bath (6.11) set at 45 °C until the butter melts. Shake well and allow to separate for no longer than 15 min. If necessary, remove the fat phase with a spatula or a glass stick (6.12)

If necessary to separate the phases, transfer the melted test portion to a sterile centrifuge tube (or melt the test portion directly in the tube) and centrifuge at a rotational frequency of 1 000 min^{-1} to 2 000 min^{-1}. It may be necessary to remove the fatty (upper) phase aseptically with a sterile tube connected to a vacuum pump. Pipette from the bottom layer.

Prepare further dilutions in accordance with 8.3.

8.2.7 **Frozen milk products** (including edible ices)

Weigh 10 g of the test sample into a sample container. Place the container in the water bath (6.11) set at 30 °C. Keep it in the water bath until the whole test portion has just melted then mix.

Prepare further dilutions in accordance with 8.3.

8.2.8 Custard, desserts and sweet cream

Weigh 10 g of the test sample into a flask (6.4) containing glass beads (6.8). Add 90 ml of diluent (5.2) at room temperature and shake to disperse.

Prepare further dilutions in accordance with 8.3.

8.2.9 Fermented milk and sour cream

Weigh 10 g of the test sample into a flask (6.4) containing glass beads (6.8). Add 90 ml of diluent (5.3.3) at pH 7,5 ± 0,2 at room temperature and shake manually. Alternatively, a peristaltic-type blender (6.2) may be used following the manufacturer's instructions. In this case, the flask should not contain any glass beads.

Prepare further dilutions in accordance with 8.3.

8.2.10 Milk-based infant foods

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it. If the test sample is in the original unopened container which is too full to permit thorough mixing, transfer it to a larger container then mix. Open the container, remove the required test portion with a spatula (6.12) and proceed as indicated below. Immediately close the container again.

Warm a bottle containing 90 ml of diluent in the water bath (6.11) to 45 °C. Weigh 10 g of the test sample into a suitable glass vessel (e.g. a beaker) and then add the powder to the dilution bottle containing a suitable diluent (5.2 or 5.3.5).

Alternatively, weigh 10 g of the test sample directly into the bottle with the diluent, prewarmed to 45 °C.

NOTE For better reconstitution, glass beads (6.8) can be helpful. If used they should be added to the bottle before sterilization.

In order to dissolve the sample, swirl slowly to wet the powder then manually shake the bottle 25 times, with a movement of about 300 mm, for about 7 s. Alternatively, a peristaltic-type blender (6.2) may be used. Replace the bottle in the water bath (6.11) for 5 min, shaking occasionally. Prepare further dilutions in accordance with 8.3.

Samples with high starch contents may cause problems because of the high viscosity of the primary dilution.

Use a diluent for general use with α-amylase (5.3.6) to reduce the viscosity of the initial solution or use twice the quantity of diluent. Take this further dilution under consideration in the following examinations.

8.3 Further decimal dilutions

For further decimal dilutions, see ISO 6887-1.

In the case of a presence-or-absence test for a microorganism in 0,1 ml or 0,1 g of test sample, it is not necessary to prepare further decimal dilutions.

Where larger volumes are required, transfer with a sterile pipette (6.7) 10 ml of the initial suspension to a bottle containing 90 ml of sterile diluent (5.2) or 11 ml of primary dilution to 99 ml of sterile diluent (5.2). In a routine procedure, if a 10^{-3} dilution is required, transfer 1 ml of the primary dilution to 99 ml of sterile diluent.

When transferring from a viscous primary dilution such as acid or rennet casein (8.2.5), rinse the pipette with diluent by aspirating several times, using the diluent in the tube used for making the decimal dilution.

WARNING — This step must be included as the primary dilution is viscous; without rinsing, the correct quantity of primary dilution would not be transferred.

When 10 ml plus 90 ml, or 11 ml plus 99 ml, have been taken, shake manually as described in 8.2.2.

8.4 Duration of the procedure

For the duration of the procedure, see ISO 6887-1.

Bibliography

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

[2] ISO 6579, *Microbiology — General guidance on methods for the detection of Salmonella.*

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