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**Milk and milk-based products — Detection
of coagulase-positive staphylococci —
Most probable number technique**

*Lait et produits à base de lait — Détection des staphylocoques à coagulase
positive — Technique du nombre le plus probable*



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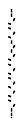
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Foreword

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

The main task of technical committees is to prepare International Standards. 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 5944 | IDF 60 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International. It is being published jointly with IDF and separately by AOAC International.

Annex A forms a normative part of this International Standard.

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, *Pathogenic contaminants*, of the Standing Committee, *Microbiological methods of analysis*, under the aegis of its project leader, Mrs M.-L. De Buyser (FR).

This edition cancels and replaces IDF 60C:1997.

Milk and milk-based products — Detection of coagulase-positive staphylococci — Most probable number technique

1 Scope

This International Standard specifies a method for the enumeration of coagulase-positive staphylococci in all types of milk and milk-based products.

It is recommended for products where staphylococci are expected to be stressed and in low numbers as, for example, in dried milk products. Coagulase-positive staphylococci will primarily be *Staphylococcus aureus* but *Staphylococcus intermedius* and some strains of *Staphylococcus hyicus* also produce free coagulase.

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

ISO 8261 | IDF 122, *Milk and milk products — Preparation of test samples and dilutions for microbiological examination*

ISO/TR 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

3 Term and definition

For the purposes of this International Standard, the following term and definition applies.

3.1

coagulase-positive staphylococci

microorganisms which form typical and/or atypical colonies on the surface of a defined selective culture medium and which show a strongly positive coagulase reaction

NOTE For the purposes of this International Standard, the confirmation of coagulase-positive staphylococci is based on a strongly positive coagulase reaction but it is recognized that some strains of coagulase-positive staphylococci give weakly positive coagulase reactions. These latter strains could be confused with other bacteria but they can be distinguished from such other bacteria by the use of additional tests such as the production of thermonuclease (see reference [3]).

4 Principle

4.1 Serial dilutions of product are inoculated into liquid selective culture medium.

4.2 Tubes are incubated at $37\text{ °C} \pm 1\text{ °C}$, anaerobically, for 24 h and 48 h, indicating the presumptive presence of coagulase-positive staphylococci if the potassium tellurite is reduced.

In this International Standard, anaerobiosis is obtained by pouring a plug of agar into each tube, but an alternative procedure is to incubate the tubes in a jar under anaerobic conditions.

4.3 The surface of selective agar medium (Baird-Parker agar medium) is inoculated with the presumptive positive liquid medium (4.2).

4.4 The plates are incubated at $37\text{ °C} \pm 1\text{ °C}$ for 24 h and 48 h, indicating the presumptive presence of coagulase-positive staphylococci if the potassium tellurite is reduced and there is a possible specific egg-yolk reaction.

4.5 A coagulase test is carried out on aliquot parts of typical and/or atypical colonies.

4.6 Alternatively, the surface of rabbit plasma fibrinogen agar is inoculated and, after appropriate incubation, the presence of coagulase-positive staphylococci is indicated in the case of colonies showing a specific rabbit plasma reaction.

4.7 The most probable number (MPN) of coagulase-positive staphylococci per gram or per millilitre of sample is calculated by comparison of confirmed dilutions (4.5 or 4.6) with most probable number tables.

5 Requirements for diluent, culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218.

In order to improve the reproducibility of the results, it is recommended that, for the preparation of the culture media, dehydrated basic components or complete dehydrated media are used. The manufacturer's instructions shall be rigorously followed. For quality assurance and performance testing of culture media, see ISO/TR 11133-1.

Use only reagents of recognized analytical grade. Use only distilled or deionized water, free from substances that might inhibit the growth of microorganisms under the test conditions.

When agar is specified, the amount used should be varied according to the manufacturer's instructions to give media of suitable firmness.

If the prepared culture media and reagents are not used immediately, they shall, unless otherwise stated, be stored in the dark at a temperature of between $+1\text{ °C}$ and $+5\text{ °C}$ for no longer than 1 month, since these conditions do not produce any change in their composition.

5.2 Diluent

See ISO 8261 | IDF 122.

Peptone/saline solution is recommended.

5.3 Liquid selective culture medium (Giolitti and Cantoni broth with Tween 80)

5.3.1 Base medium

5.3.1.1 Composition

	a) Double-strength medium	b) Single-strength medium
Tryptone	20,0 g	10,0 g
Meat extract	10,0 g	5,0 g
Yeast extract	10,0 g	5,0 g
Lithium chloride	10,0 g	5,0 g
Mannitol	40,0 g	20,0 g
Sodium chloride	10,0 g	5,0 g
Glycine	2,4 g	1,2 g
Sodium pyruvate	6,0 g	3,0 g
Tween 80	2,0 g	1,0 g
Water	1 000 ml	1 000 ml

5.3.1.2 Preparation

Dissolve the ingredients in the water, with heating and shaking to obtain complete dissolution. Cool to room temperature and adjust the pH to $6,9 \pm 0,2$.

Dispense the medium in quantities of 10 ml into tubes (6.6) of dimensions 16 mm × 160 mm in the case of single-strength medium and into tubes of dimensions 20 mm × 200 mm in the case of double-strength medium.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

5.3.2 Potassium tellurite solution

5.3.2.1 Composition

Potassium tellurite (K_2TeO_3)	1,0 g
Water	100 ml

5.3.2.2 Preparation

Dissolve the potassium tellurite in the water with minimal heating. Sterilize by filtration.

The solution may be stored for several months at between +1 °C and +5 °C.

5.3.3 Complete medium

Shortly before use, heat the base medium (5.3.1) for 15 min at 100 °C to expel air. Cool in the water bath (6.8) to (44 to 47) °C and aseptically add the potassium tellurite solution (5.3.2) using 0,1 ml for single-strength medium and 0,2 ml for double-strength medium.

Commercially available medium conforming to the above formulation may be used.

5.4 Agar solution

5.4.1 Composition

Agar	20 g
Water	1 000 ml

5.4.2 Preparation

Dissolve the agar in the water by boiling. Sterilize for 15 min in the autoclave (6.1) set at 121 °C. Before use, cool in the water bath (6.8) to (44 to 47) °C.

5.5 Selective agar medium (Baird-Parker agar medium)

5.5.1 Base medium

5.5.1.1 Composition

Tryptone	10,0 g
Yeast extract	1,0 g
Meat extract	5,0 g
Sodium pyruvate	10,0 g
Glycine	12,0 g
Lithium chloride	5,0 g
Agar	12 g to 20,0 g ^a
Water	950 ml

^a Depending on the gel strength of the agar.

5.5.1.2 Preparation

Dissolve the components or the complete agar base in the water by boiling. Adjust the pH so that after sterilization it is $7,2 \pm 0,2$ at 25 °C.

Transfer the medium in quantities of 95 ml to flasks or bottles of capacity not more than 300 ml. Sterilize the medium for 15 min in the autoclave (6.1) set at 121 °C.

5.5.2 Egg-yolk emulsion

Use commercially available egg-yolk emulsion with a concentration of approximately 20 % (volume fraction).

If a commercial emulsion is not available, wash the shells of fresh hens' eggs in a liquid detergent, rinse in running water and dip into ethanol 70 % (volume fraction) and drain. Using aseptic procedures, break each egg into a Petri dish (6.4) and separate the white from the yolk by tilting the dish with the lid slightly open. Add the yolks to a sterile measuring cylinder with four times their volume of sterile water and mix thoroughly.

Heat the mixture in the water bath (6.8) at (44 to 47) °C for 2 h then leave for 18 h to 24 h at +1 °C to +5 °C to allow a precipitate to form. Collect the supernatant emulsion aseptically.

The emulsion may be stored at +1 °C to +5 °C for no longer than 72 h.

5.5.3 Complete medium

5.5.3.1 Composition

Base medium (5.5.1)	95 ml
Potassium tellurite solution (5.3.2)	1,0 ml
Egg-yolk emulsion (5.5.2)	5,0 ml

5.5.3.2 Preparation

Melt the base medium, then cool it in the water bath (6.8) to (44 to 47) °C . Add the other liquids, prewarmed in the water bath (6.8), mixing well after each addition.

Commercially available medium conforming to the above formulation may be used.

5.5.4 Preparation of agar plates

Transfer 12 ml to 15 ml of the complete medium (5.5.3), cooled in the water bath (6.8) to (44 to 47) °C, to sterile Petri dishes (6.4). Allow it to solidify. Prior to drying, the plates may be stored at +1 °C to +5 °C for up to 24 h.

For commercially prepared, ready-to-use plates, follow the manufacturer's instructions.

Dry the plates in a drying cabinet, oven or incubator (6.3) set at 45 °C for 30 min or set at 37 °C for 1 h with the lids off and the agar surface downwards. The plates may also be dried for 30 min in a laminar airflow cabinet with half-open lids.

5.6 Alternative selective agar medium (Rabbit plasma fibrinogen agar medium)

5.6.1 Base medium

Prepare the base medium as mentioned in 5.5.1 except that the medium should be distributed in quantities of 90 ml to flasks or bottles.

5.6.2 Solutions

5.6.2.1 Bovine fibrinogen solution

5.6.2.1.1 Composition

Bovine fibrinogen	5 g to 7 g ^a
Sterile water	100 ml
^a Depending on the purity of the bovine fibrinogen.	

5.6.2.1.2 Preparation

Using aseptic precautions, dissolve the bovine fibrinogen in the water immediately before use.

5.6.2.2 Plasma-trypsin inhibitor solution

5.6.2.2.1 Composition

Coagulase plasma EDTA	30 ml
Trypsin inhibitor	30 mg

5.6.2.2.2 Preparation

Using aseptic precautions, prepare this solution immediately before use.

5.6.3 Complete medium

5.6.3.1 Composition

Base medium (5.6.1)	90 ml
Potassium tellurite solution (5.3.2)	0,25 ml
Bovine fibrinogen solution (5.6.2.1)	7,5 ml
Plasma-trypsin inhibitor solution (5.6.2.2)	2,5 ml

5.6.3.2 Preparation

Melt the base medium and then cool it in the water bath (6.8) to (44 to 47) °C or according to the manufacturer's instructions. Add the other liquids, prewarmed in the water bath (6.8). Mix well after each addition. Immediately after preparation, transfer the medium to Petri dishes as indicated in 5.6.3.3.

Commercially available medium conforming to the above formulation may be used. Before use, test each batch of rabbit plasma fibrinogen supplement. Respect rigorously the manufacturer's instructions for preparation of supplement and complete medium otherwise this medium could completely lose its properties.

5.6.3.3 Preparation of agar plates

Transfer 12 ml of the complete medium to sterile Petri dishes (6.4). Allow it to solidify. Prior to drying, the plates may be stored at +1 °C to +5 °C for up to 24 h, or according to manufacturer's instructions.

Dry the plates in a drying cabinet, oven or incubator (6.3) set at 45 °C for 30 min or set at 37 °C for 1 h with the lids off and the agar surface downwards. The plates may also be dried for 30 min in a laminar airflow cabinet with half-open lids.

5.7 Brain-heart infusion broth

5.7.1 Composition

Peptone	10,0 g
Dehydrated calf brain infusion	12,5 g
Dehydrated beef heart infusion	5,0 g
Glucose	2,0 g
Sodium chloride	5,0 g
Disodium hydrogenphosphate (Na ₂ HPO ₄)	2,5 g
Water	1 000 ml

5.7.2 Preparation

Dissolve the components or the complete medium in the water by boiling. Adjust the pH so that after sterilization it is $7,4 \pm 0,2$ at 25 °C. Transfer the culture medium to tubes or bottles in quantities of 10 ml.

Sterilize the medium for 15 min in the autoclave (6.1) set at 121 °C.

The medium may be stored for several months at between +1 °C and +5 °C.

5.8 Rabbit plasma

Use commercially available dehydrated rabbit plasma. Only plasma with EDTA as anticoagulant may be used. Rehydrate it according to the manufacturer's instructions. If dehydrated rabbit plasma is not available, dilute fresh sterile rabbit plasma (one volume of plasma to three volumes of sterile water). If not already present, add EDTA solution to give 0,1 % EDTA in the rehydrated plasma.

Before use, test each batch of plasma with weakly and strongly coagulase-positive strains of *Staphylococcus aureus* and strains of coagulase-negative staphylococci.

6 Apparatus

Sterilize all apparatus that will be in contact with the test sample, the diluent, the dilutions or the culture media in accordance with ISO 7218.

Disposable apparatus is an acceptable alternative to re-usable glassware if it has similar specifications.

Usual microbiological laboratory apparatus, the apparatus required for the preparation of test samples and dilutions as specified in ISO 7218 and, in particular, the following.

6.1 Autoclave, capable of being maintained at $121 \text{ °C} \pm 1 \text{ °C}$.

6.2 Incubator, capable of operating at $37 \text{ °C} \pm 1 \text{ °C}$.

6.3 Drying cabinet, oven or incubator, ventilated (for drying the surface of agar plates), capable of being maintained at 45 °C or 37 °C. A laminar airflow cabinet may also be used.

6.4 Petri dishes, made of glass or plastic, of 90 mm diameter.

6.5 Loop of platinum-iridium, nickel-chromium or plastic, of approximately 3 mm diameter, and stab inoculation wires of the same material.

6.6 Test tubes, of dimensions 16 mm × 160 mm, 20 mm × 200 mm and 10 mm × 75 mm.

6.7 Graduated pipettes, calibrated for bacteriological use only, of 1 ml nominal capacity, graduated in 0,1 ml divisions and with an outflow opening of 2 mm to 3 mm.

6.8 Water bath, capable of operating at between 44 °C and 47 °C.

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.

8 Preparation of test sample

Follow the procedures given in ISO 8261 | IDF 122.

9 Procedure

9.1 Test portion, initial suspension and further dilutions

Prepare the test portion, initial suspension (primary dilution) and further decimal dilutions in accordance with ISO 8261 | IDF 122.

9.2 Duration of the procedure

See ISO 8261 | IDF 122.

9.3 Inoculation of liquid selective culture medium

9.3.1 Take three tubes of double-strength medium [5.3.1.1a)] with potassium tellurite solution added (5.3.3) and transfer to each of these tubes 10 ml of the test sample for liquid products or 10 ml of the primary dilution (equal to 1 g of sample) for other products.

9.3.2 Then take three tubes of single-strength medium [5.3.1.1b)] with potassium tellurite solution added (5.3.3) and transfer to each of these tubes 1 ml of the test sample for liquid products or 1 ml of the primary dilution (equal to 0,1 g of sample) for other products.

9.3.3 For each of the subsequent dilutions (equal to 0,1 ml, 0,01 ml, and 0,001 ml of the test sample for liquid products or equal to 0,01 g, 0,001 g and 0,000 1 g of sample for other products) proceed as specified in 9.3.2, using a fresh pipette for each dilution.

9.3.4 Carefully mix the inoculum and medium, in each case avoiding the introduction of air.

9.3.5 Carefully pour a plug of agar (5.4), cooled in the water bath (6.8) to between 44 °C and 47 °C, onto the top of the medium in each inoculated tube and allow it to solidify to form a seal.

9.4 Incubation

Incubate the inoculated tubes of double-strength medium (9.3.1) and single-strength medium (9.3.2 and 9.3.3) in the incubator (6.2) set at 37 °C for 24 h. Subculture any tubes showing any blackening or black precipitate as indicated in 9.5.

Incubate the remainder of the inoculated tubes for a further 24 h and subculture all tubes whether or not they develop a black precipitate after 48 h, as indicated in 9.5.

9.5 Subculture

9.5.1 Remove the plug of agar. Use a sterile spatula to cut the agar plug across two diameters (at right angles) and, if necessary, insert the spatula around the plug to release it from the glass wall of the tube. Agitate the tube to cause the pieces of plug to fall to the bottom of the tube and to ensure an even suspension of the culture.

9.5.2 With a sterile loop (6.5), spread about 0,001 ml of the medium (9.5.1) onto the surface of Baird-Parker medium plates (5.5) or of rabbit plasma fibrinogen agar plates (5.6).

9.5.3 Invert the prepared dishes (9.5.2) and place them in the incubator (6.2) set at 37 °C for 24 h and 48 h.

9.6 Selection of plates and interpretation

9.6.1 Baird-Parker agar medium

After incubation for 24 h, mark on the bottom of the plates the positions of any typical colonies present.

Re-incubate all plates in the incubator (6.2) set at 37 °C for a further 24 h and mark any newly developed typical colonies; also mark any atypical colonies present.

NOTE Atypical colonies have one of the following morphologies:

- a) black, shiny, narrow white margin with the clear zone absent or scarcely visible;
- b) less black than a), but with a rough, dry texture.

Atypical colonies are formed frequently by strains of coagulase-positive staphylococci contaminating dairy products.

Select for confirmation (9.7) three typical and/or atypical colonies, as the case may be, from the plate corresponding to each tube (that is, presumptive coagulase-positive staphylococci).

9.6.2 Rabbit plasma fibrinogen agar medium

After incubation for 24 h to 48 h, coagulase-positive staphylococci form black, grey or even white colonies surrounded by an opaque or cloudy zone indicating coagulase activity.

Take the reaction as positive when the presence of at least one colony indicating coagulase activity is seen.

NOTE Rabbit plasma fibrinogen agar is based on a coagulase reaction. Therefore, no further confirmation of coagulase activity (9.7) is normally necessary.

9.7 Confirmation (coagulase test)

From the surface of each selected colony (9.6.1), remove an inoculum with a sterile wire and transfer it to a tube or bottle of brain-heart infusion broth (5.7). Incubate in the incubator (6.2) set at 37 °C for 20 h to 24 h.

Add 0,1 ml of each culture aseptically to 0,3 ml of the rabbit plasma (5.8) (unless the manufacturer specifies other amounts) in sterile tubes of dimensions 10 mm × 75 mm. Incubate in the incubator (6.2) set at 37 °C. Examine for clotting of the plasma after 4 h to 6 h and, if still negative after 6 h, repeat after 24 h.

Consider the coagulase test to be positive if the volume of clot occupies more than half of the original liquid volume.

As a control, add 0,1 ml of sterile brain-heart infusion broth (5.7) to the recommended quantity of rabbit plasma (5.8) and incubate without inoculation. For the test to be valid, the control plasma shall show no signs of clotting.

Record as positive each tube from which at least one colony is confirmed as coagulase positive.

10 Calculation and expression of results

10.1 Selection of dilutions

10.1.1 For each dilution of liquid selective culture medium inoculated (9.3) record the number of tubes in which the presence of coagulase-positive staphylococci has been confirmed (9.6 and 9.7). Designate these as positive tubes.

Select three consecutive dilutions in accordance with 10.1.2, 10.1.3 or 10.1.4 to obtain the MPN index.

NOTE The initial suspension and, if liquid, the test sample are considered as dilutions.

10.1.2 In the case where only three dilutions were made, use those three dilutions to obtain the MPN index.

10.1.3 In the case where more than three dilutions were made, the selection of three of these gives combinations with different degrees of probability. This can be expressed in categories (Table A.1). An explanation of these categories is given in Table A.2.

10.1.4 Select the combination of three consecutive dilutions with category 1, using Table A.1, to obtain the MPN index. If more than one combination with category 1 is obtained, use the one with the highest number of positive tubes. If no combination with category 1 is available, use the one with category 2; if more than one combination with category 2 is obtained, use the one with the highest number of positive tubes (see Table 1 for examples).

Table 1 — Examples of the selection of positive results for the calculation of the MPN

Example	Number of positive tubes obtained from three incubated tubes for the following quantities of samples per tube ^a					MPN ^b		
	Liquid product	10 ml	1 ml	10 ⁻¹ ml	10 ⁻² ml	10 ⁻³ ml	Liquid product ml ⁻¹	Other products g ⁻¹
	Other products	1 g	10 ⁻¹ g	10 ⁻² g	10 ⁻³ g	10 ⁻⁴ g		
1	<u>3</u>	<u>3</u>	<u>2</u>	1	0		1,1 × 10	1,1 × 10 ²
2	3	<u>3</u>	<u>3</u>	<u>0</u>			2,4 × 10	2,4 × 10 ²
3	2	2	<u>1</u>	<u>1</u>	<u>0</u>		7,4	7,4 × 10
4	<u>3</u>	<u>3</u>	<u>0</u>	0	0		2,4	2,4 × 10
5	<u>2</u>	<u>2</u>	<u>0</u>	1	0		2,1 × 10 ⁻¹	2,1

^a Underlined: combination selected.
^b Calculated using the MPN index (Table A.1).

10.2 Determination of MPN index

Determine the MPN index which corresponds to the dilutions selected (10.1), using Table A.1.

10.3 Calculation of the most probable number (MPN)

Obtain the most probable number (MPN) of coagulase-positive staphylococci per gram or per millilitre of product by multiplying the MPN index (10.2) by the reciprocal of the lowest dilution selected (that is, the one having the highest sample content).

When the lowest dilution selected corresponds to the tubes prepared with double-strength medium (inoculation with 10 ml) and when the test sample is a liquid product, first divide the MPN index by 10.

NOTE A division of the MPN index by 10 is only necessary with liquid products where 10 ml of the test sample are transferred to the tube with double-strength medium. In the case of other products, 10 ml of the initial suspension (primary dilution), containing 1 g of test sample, are transferred.

10.4 Expression of results

Express the result as the most probable number (MPN) of coagulase-positive staphylococci per gram or millilitre of product, expressed as a number between 1,0 and 9,9 multiplied by 10^x, where x is the appropriate power of 10 (see Table 1).

If the MPN is lower than 0,3 coagulase-positive staphylococci per gram or per millilitre, and if the appropriate procedure for a low number of coagulase-positive staphylococci was used, express the result in the following way: "No coagulase-positive staphylococci in 1 g or 1 ml of the product".

11 Precision

It is well known that wide variations in results can occur with the MPN technique. Results obtained from this method should therefore be used with caution. The specified confidence limits are based entirely on a random distribution of results. Other sources of variation, which may sometimes be of even greater importance, do not enter in the MPN estimate. To facilitate the recognition of such effects, use the categories in Table A.1. These summarize the possible tube combinations according the probability of their occurrence.

12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operational details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result;
- the test result obtained.

Annex A
(normative)

Determination of most probable number

Table A.1 — MPN indexes and confidence limits

Number of positive results			MPN ^a Index	Category ^b	Confidence limits (95 %) ^{a, c}	
					Lower	Upper
0	0	0	< 0,30		0,00	0,94
0	0	1	0,30	3	0,01	0,95
0	1	0	0,30	2	0,01	1,00
0	1	1	0,61	0	0,12	1,70
0	2	0	0,62	3	0,12	1,70
0	3	0	0,94	0	0,35	3,50
1	0	0	0,36	1	0,02	1,70
1	0	1	0,72	2	0,12	1,70
1	0	2	1,1	0	0,4	3,5
1	1	0	0,74	1	0,13	2,00
1	1	1	1,1	3	0,4	3,5
1	2	0	1,1	2	0,4	3,5
1	2	1	1,5	3	0,5	3,8
1	3	0	1,6	3	0,5	3,8
2	0	0	0,92	1	0,15	3,50
2	0	1	1,4	2	0,4	3,5
2	0	2	2,0	0	0,5	3,8
2	1	0	1,5	1	0,4	3,8
2	1	1	2,0	2	0,5	3,8
2	1	2	2,7	0	0,9	9,4
2	2	0	2,1	1	0,5	4,0
2	2	1	2,8	3	0,9	9,4
2	2	2	3,5	0	0,9	9,4
2	3	0	2,9	3	0,9	9,4
2	3	1	3,6	0	0,9	9,4
3	0	0	2,3	1	0,5	9,4
3	0	1	3,8	1	0,9	10,4
3	0	2	6,4	3	1,6	18,1
3	1	0	4,3	1	0,9	18,1
3	1	1	7,5	1	1,7	19,9
3	1	2	12	3	3	36
3	1	3	16	0	3	38
3	2	0	9,3	1	1,8	36,0
3	2	1	15	1	3	38
3	2	2	21	2	3	40
3	2	3	29	3	9	99
3	3	0	24	1	4	99
3	3	1	46	1	9	198
3	3	2	110	1	20	400
3	3	3	> 110			

^a From reference [2].

^b See Table A.2.

^c The confidence limits given in this Table are meant only to provide some idea of the influence of statistical variations on results. There will always also be other sources of variation, which may sometimes be even more important.

Table A.2 — Explanation of categories for results

Category ^a	Definition
1	When the number of bacteria in the sample is equal to the MPN found, the result is one of those that have the greatest chance of being obtained. There is only at most 5 % chance of obtaining a result that is less likely than the least likely one in this category.
2	When the number of bacteria in the sample is equal to the MPN found, the result is one of those that have less chance of being obtained than even the least likely one in category 1. But there is only at most 1 % chance of obtaining a result that is less likely than the least likely one in this category.
3	When the number of bacteria in the sample is equal to the MPN found, the result is one of those that have less chance of being obtained than even the least likely one in category 2. But there is only at most 0,1 % chance of obtaining a result that is less likely than the least likely one in this category.
0	When the number of bacteria in the sample is equal to the MPN found, the result is one of those that have less chance of being obtained than even the least likely one in category 3. There is only a chance of 0,1 % of obtaining a result in this category, without anything being wrong.

^a Before starting testing it should be decided which category will be acceptable, that is, only 1, 1 and 2 or even 1, 2 and 3. When the decision to be taken on the basis of the result is of great importance, only category 1 or at most category 1 and 2 results should be accepted. Category 0 results should be considered with great suspicion.

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