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PROFESSIONAL STANDARD

OF THE PEOPLE'S REPUBLIC OF CHINA

NY/T 1331-2007

**Enumeration of Colony of Psychrotrophic
Microorganisms, Total Aerobic Bacterial
Spores and Thermophilic Aerobic Bacterial
Spores in Milk and Dairy Products**

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Foreword

This Standard is drafted by reference to "Milk - Enumeration of Colony-forming Units of Psychrotrophic Micro-organisms - Colony-Count Technique at 6.5°C" (ISO 6730: 1992, English version), "Milk and Dairy Products-Determination of the Content of Spores of Mesophilic Aerobic Sporeforming Bacteria" (NEN 6813: 1999, Dutch version) and "Milk and Dairy Products - Determination of the Content of Thermophilic Aerobic Sporeforming Bacteria" (NEN 6809: 1999, Dutch version). This Standard is not equivalent to ISO 6730: 1992, NEN 6813: 1999, NEN 6809: 1999.

This Standard was proposed by the Ministry of Agriculture of the People's Republic of China.

This Standard is under the jurisdiction of Animal Husbandry of Standardization Administration of China.

Drafting organization of this Standard: Ministry of Agriculture Dairy Quality Supervision and Testing Center

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Enumeration of Colony of Psychrotrophic Microorganisms, Total Aerobic Bacterial Spores and Thermophilic Aerobic Bacterial Spores in Milk and Dairy Products

Part I: Enumeration of Colony of Psychrotrophic Microorganisms in Milk

1 Scope

This Part specifies the test method for the enumeration of colony of psychrotrophic microorganisms in milk.

This Part is applicable to the enumeration of colony of psychrotrophic microorganisms in raw and fresh milk and pasteurized milk.

2 Normative References

The following normative documents contain provisions which, through reference by this Part, constitute the provisions of this Part. For dated reference, subsequent amendments (excluding the corrected contents) or revisions do not apply to this Part. However, parties who enter into agreements based on this Standard are encouraged to investigate the possibility of applying the most recent editions of the relevant standards indicated below. For any undated references, the latest edition of the document applies to this Part.

GB/T 4789.1 Microbiological Examination of Food Hygiene - General Principles

GB/T 4789.28-2003 Microbiological Examination of Food Hygiene - Staining Methods, Culture Mediums and Reagents

3 Terms and Definitions

The following terms and definitions are applicable to this Part.

3.1 Psychrotrophic microorganisms

Under condition of 6.5°C, with aerobic incubation of 10d, the bacteria, yeasts and mildews forming countable colonies on MPC agar plate.

3.2 MPC agar

Milk plate count agar

4 Principle

Incubate the sample in the milk plate count agar, at 6.5°C for 10d, then count the number of psychrotrophic microorganisms per milliliter through colony counting.

5 Diluent and Culture Medium

5.1 Peptone salt solution

5.1.1 Components

Enzyme hydrolyzed casein peptone	1.0g
Sodium chloride	8.5g
Distilled water	1000mL

5.1.2 Preparation Method

shall not be greater than 30% of the lower result; with the premise of - situations with greater-than-30% of the lower result shall not exceed 5%.

Part II: Enumeration of Colony of Aerobic Bacterial Spores

1 Scope

This Part specifies the test method for enumeration of colony of aerobic bacterial spores in milk and dairy products.

This Part is applicable to the enumeration of colony of aerobic bacterial spores in raw and fresh milk and its products.

2 Normative References

The following normative documents contain provisions which, through reference by this Part, constitute the provisions of this Part. For dated reference, subsequent amendments (excluding the corrected contents) or revisions do not apply to this Part. However, parties who enter into agreements based on this Standard are encouraged to investigate the possibility of applying the most recent editions of the relevant standards indicated below. For any undated references, the latest edition of the document applies to this Part.

GB/T 4789.1 Microbiological Examination of Food Hygiene - General Principles

GB/T 4789.28 - 2003 Microbiological Examination of Food Hygiene - Staining Methods, Culture Mediums and Reagents

3 Terms and Definitions

The following terms and definitions are applicable to this Part.

3.1 Total aerobic bacterial spores

Under aerobic condition, after 80℃ heat treatment for 10min, the number of CFU of microorganisms on MPC agar plate with incubation of 48h at 36℃.

3.2 Milk plate count agar

4 Principle

Take a certain amount of liquid sample or the primary diluent of sample. Heat it at 80℃ for 10min. Incubate it in the milk plate count agar at 36℃ for 48h. Calculate the total aerobic bacterial spores per milliliter through colony counting.

5 Diluent and Culture Medium

5.1 Peptone salt solution

5.1.1 Components

Enzyme hydrolyzed casein peptone	1.0g
Sodium chloride	8.5g
Distilled water	1000mL

5.1.2 Preparation method

Dissolve the enzyme hydrolyzed casein peptone and sodium chloride into distilled water. Calculate the pH value to 7.0 ± 0.1 with sodium hydroxide solution with mass fraction of 15%. Pour into test tubes and flasks, 9mL per tube, 90mL or 225mL per flask, relative error less than or equal to $\pm 2\%$. After sealed, $121^\circ\text{C} \pm 1^\circ\text{C}$ autoclave for 15min.

5.2 Phosphate buffer: prepared in accordance with 3.22 of GB/T 4789.28-2003.

5.3 Milk plate count agar culture medium

5.3.1 Components

Tryptone	5.0g
Yeast extract	2.5g

further 10-times diluent (10^{-1} diluent for liquid sample or 10^{-2} diluent for solid sample).

9.4 Inoculation and incubation

9.4.1 According to the estimated spore contents in the sample, select proper dilutions. Through proper selection, there is at least one petri dish containing 10~150 colonies after incubation. While the 10-times dilution is being carried out, simultaneously use the sterile pipette to suck and transfer 1mL such diluent into the sterile petri dishes. Each dilution inoculates two dishes.

Notes: Since the milk or dairy products only contains a small amount of thermophilic aerobic bacterial spores per milliliter (gram), the following measures may be taken for the enumeration of their quantity:

- Inoculate sufficient heat treated liquid sample or primary diluent in multiple petri dishes;
- Use bigger petri dish to inoculate more than 1mL heat treated liquid sample or primary diluent;

Adjustment of test method must be indicated in the result report.

9.4.2 Immediately pour 12~15mL DTA culture medium (melted and cooled to $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$) into each petri dish.

9.4.3 Carefully mix the sample inoculum with the culture medium by rotating the petri dish, and allow the mixture to solidify by leaving the petri dish on a horizontal surface. The time taken between the end of heat treatment and the mixing of sample inoculum with culture medium shall not exceed 15min.

9.4.4 Conduct a blank test with 1mL diluent when inoculating.

9.4.5 After the agar is solidified, pile and reversely place the petri dishes in the incubator (see Section 6.2), at $55^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for $48\text{h} \pm 2\text{h}$.

Notes: the following measures may be taken in order to avoid uncountable colonies due to spreading.

- After the solidification of the mixture in petri dish, add a new layer of such liquid culture medium to it.
- After the solidification of the mixture in petri dish, overturn the petri dish, put a piece of filter paper in the petri dish

cover, and then drip a few drops of glycerol on such paper.

9.5 Colony counting

9.5.1 Use visual inspection for plate colony counting, magnifier may also be used if required.

9.5.2 Spreading colonies shall be considered as single colonies. If less than 1/4 of the plate surface is overgrown by spreading colonies, count the colonies on the unaffected part of the plate and calculate the corresponding number for the entire plate. If more than 1/4 of the plate surface is overgrown by spreading colonies, counting shall not be taken.

10 Result Calculation

10.1 Retain those dishes containing 10~150 colonies.

Calculate the number of CFU/mL of thermophilic aerobic bacterial spores in the sample using Formula (3):

$$N = \frac{\sum c}{(n_1 + 0.1n_2)d} \quad (3)$$

Where:

N - The number of thermophilic aerobic bacterial spores in the sample, CFU/mL(g);

$\sum c$ - The sum of colonies counted on all the retained dishes;

n_1 - In the 1st dilution, the number of retained dishes which contain 10 to 150 colonies;

n_2 - In the 2nd dilution, the number of retained dishes which contain 10 to 150 colonies;

d - The dilution factor corresponding to the 1st dilution.

References and Original Chinese Documents

[1] NY/T 1331-2007 Enumeration of Colony of Psychrotrophic Microorganisms, Total Aerobic Bacterial Spores and Thermophilic Aerobic Bacterial Spores in Milk and Dairy Products.

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