GB

NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.15-2016

National Food Safety Standard – Food Microbiological Examination: Enumeration of Moulds and Yeasts

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Foreword

This Standard replaced GB 4789.15-2010 *National Food Safety Standard – Food Microbiological Examination: Enumeration of Moulds and Yeasts*; and SN/T 2552.3-2010 *Microbiological Examination Method for Milk and Milk Products Hygiene – Part 3: Colony-Count Method of Yeast and Moulds*.

Compared with GB 4789.15-2010, this Standard has the major changes as follows:

--- Modify the equipment and materials;

--- Modify the culture medium and reagents;

--- Modify the examination procedures and operation steps;

--- Modify the results and report;

--- Modify the Appendix A;

--- Modify the Appendix B into the second method.

National Food Safety Standard – Food Microbiological Examination: Enumeration of Moulds and Yeasts

1 Scope

This Standard specifies the enumeration method of moulds and yeasts in the food.

The first method in this Standard is applicable to the enumeration of moulds and yeasts in various foods; while the second method is applicable to the enumeration of moulds in the canned tomato sauce and tomato juice.

2 Equipment and materials

In addition to the biological laboratory routine sterilization and cultivating equipment, other equipment and materials are as follows:

2.1 Incubator: 28°C±1°C.

2.2 Beat-type homogenizer and homogeneous bag.

2.3 Electronic balance: sensitivity of 0.1g.

2.4 Sterile conical flask: capacity of 500mL.

2.5 Sterile pipette: 1mL (with 0.01mL scale), 10mL (with 0.1mL scale).

2.6 Sterile test tube: 18mm × 180mm.

2.7 Vortex mixer.

2.8 Sterile flat plate: diameter of 90mm.

2.9 Constant temperature water batch: 46°C±1°C.

2.10 Microscope: 10× ~ 100×.

2.11 Micro-pipettor and tip: 1.0mL.

2.12 Refractometer.

bottle) containing sterile diluent (distilled water or normal saline or phosphate buffering solution) or into the sterile homogeneous bag; sufficiently shake, or use beat-type homogenizer to beat for 1min ~ 2min; and 1:10 sample homogenous solution is prepared.

5.1.3 Take 1mL of 1:10 sample homogenous solution to inject into the test tube containing 9mL of sterile diluent; change another 1mL sterile pipette to blow and absorb repeatedly; or mix evenly on the vortex mixer; such solution is 1:100 sample homogenous solution.

5.1.4 Operate as per 5.1.3, prepare the 10 times incremental serial sample homogenous solution. Once incrementally dilute, replace 1 piece of 1mL sterile pipette.

5.1.5 According to the evaluation of the sample pollution, select the sample homogenous solution (liquid sample can include the stock solution) with 2 ~ 3 appropriate dilution; when performing the 10 times incremental dilution, absorb 1mL of sample homogenous solution for each dilution, and place into 2 sterile flat plates, respectively. Meanwhile, take 1mL of sterile diluent and add into 2 sterile flat plates to make the blank control.

5.1.6 Timely cool off the 20mL ~ 25mL of potato dextrose agar or rose Bengal agar (can place into a 46°C±1°C constant temperature water bath for thermal insulation) to 46°C, then pour into the flat plate; rotate the flat plate to make it mix evenly. Place on the horizontal platform till the culture medium is completely solidified.

5.2 Cultivation

After agar solidification, upright the flat plat, and place into 28°C±1°C incubator for cultivation; observe and record the cultivation results to the first 5d.

5.3 Colony counting

Perform visual examination; if necessary, use magnifier or low power lens to record dilution factor, and corresponding number of moulds and yeast colonies. It is expressed by the Colony-Forming Unit (CFU).

Select the flat plate with number of colonies 10CFU ~ 150CFU, count the moulds and yeasts, respectively according to the colony morphology. When the moulds spreading and growing over the whole flat plate, it can be recorded as the colony spread.

6 Results and Report

6.1 Results

6.1.1 Calculate the average value of two flat plate colonies at the same dilution, then multiply the average value by the corresponding dilution factor.

Appendix A

Culture Medium and Reagents

A.1 Normal saline

A.1.1 Components

Sodium chloride	8.5g
Distilled water	1000mL

A.1.2 Preparation

Add sodium chloride into 1000mL of distilled water, mix till it is fully dissolved; after packaging separately, sterilize for 15min at 121°C, then backup.

A.2 Potato dextrose agar

A.2.1 Components

Potato (peeled and cut into blocks)	300g
Dextrose	20.0g
Agar	20.0g
Chloromycetin	0.1g
Distilled water	1000mL

A.2.2 Preparation

Peel the potato and cut it into blocks; add 1000mL of distilled water, and boil for 10min ~ 20min. Use gauze to filter, add distilled water to 1000mL. Add dextrose and agar, heating for dissolution; after packaging separately, sterilize for 15min at 121°C, then backup.

A.3 Rose Bengal agar

Peptone	5.0g
Dextrose	10.0g
Potassium dihydrogen phosphate	1.0g
Magnesium sulfate (anhydrous)	0.5g

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