



**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**

食品安全国家标准 食品微生物学检验 霉菌和酵母计数

(English Translation)

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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-

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 Method 2.

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 Method 1 in this Standard is applicable to the enumeration of moulds and yeasts in various foods; while the Method 2 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.

4 Examination procedures

The examination procedures of mould and yeast plate counting method can refer to Figure 1.

5.1.1 Solid and semi-solid samples. weigh 25g of sample; add 225mL of sterile diluent (distilled water or normal saline or phosphate buffering solution), sufficiently shake; or use beat-type homogenizer to beat for 1min ~ 2min; then 1.10 sample homogenous solution is prepared.

5.1.2 Liquid sample. use the sterile pipette to absorb 25mL of sample into 225mL appropriate container (can pre-set appropriate amount of sterile glass beads in the Sample Examination

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.

5.1.6 Timely cool off the 20mL ~ 25mL of potato dextrose agar or rose Bengal agar

(can place into a $46^{\circ}\text{C}\pm 1^{\circ}\text{C}$ 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.

5.2 Cultivation

After agar solidification, upright the flat plat, and place into $28^{\circ}\text{C}\pm 1^{\circ}\text{C}$ 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).

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.

6.1.2 If the number of colonies on two dilution flat plate is in 10CFU ~ 150CFU, calculate the corresponding values as per the provisions of GB 4789.2.

6.1.3 If the number of colonies on all flat plates are greater than 150CFU, count the

flat plate with the maximum dilution; other plates are recorded as unacceptable; the result shall be calculated through the average number of colonies multiply by the maximum dilution factor.

6.1.6 If the number of colonies on the flat plate with all dilution aren't in 10CFU ~ 150CFU, thereof, one part is less than 10CFU or greater than 150CFU, then it shall be calculated through the average number of colonies closest to 10CFU or 150CFU multiply by the dilution factor.

6.2.1 The number of colonies shall be rounded off as per the "tetrahedral hybridization" principle. If the number of colonies are within 10, take one effective digit to report; if the number of colonies are in 10 ~ 100, take two effective digits to report.

6.2.2 When the number of colonies are greater than or equal to 100, after the first three digits are rounded off as per the "tetrahedral hybridization" principle, the result shall be expressed by the first two digits plus "0" replacing the following digit capacities; or expressed by the index number of 10;

6.2.3 If the colony appears on the blank control flat plate, then such test results are
invalid.

6.2.4 The weight sampling shall be reported in unit of CFU/g; while the volume sampling shall be reported in unit of CFU/mL; report, or respectively report the number of moulds and/or yeasts.

7.1 Preparation of the sample for examination. take appropriate amount of sample

for examination; add distilled water to dilute to the refractive index to be 1.3447 ~

7.2 Microscope calibration of standard field of view. magnify the microscope for 90x

~ 125x, adjust the standard field of view; so that its diameter is 1.382mm.

7.3 Smear. 7.4 Observation. place the prepared slide under the standard field of view of

microscope to observe. Generally, one sample for examination shall be observed for

50 fields of view each person. The same sample for examination shall be observed by

two persons.