National Standard of the People's Republic of China

GB4789.15—2016

National Standards For Food Safety

Food Microbiological Analysis
Mould and Yeast Count

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Forward

This standard replaces GB 4789.15-2010 “National Standard for Food Safety, Food Microbiological Test for mold and yeast count” and SN/T 2552.3-2010 "Hygienic microbiological test methods for milk and dairy products - Part 3: Yeast and mold colony count".The main changes of this standard compared with GB 4789.15-2010 are as follows

——Modified equipment and materials;

——Modified media and reagents;

——Modified the test procedure and operation procedure

——Modified the results and reports;

——Modified the Appendix A:

——Appendix B is amended as the second Law.

1Scope

This standard specifies the method of detecting molds and yeasts in foods.

The first method applies to determination of molds and yeasts in all foods. The second method applies to determination of molds in canned tomato sauce, tomato juice.

2 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratory, other equipment and materials are as follows:

2.1 Constant temperature incubator: 38℃ ±1℃.

2.2 Slapping homogenizer and homogenizer bag.

2.3 Balance: 0.1g of sensibility.

2.4 Sterile conical flask: 500mL.

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

2.6 Sterile test tube: 18mm ×180mm

2.7 Turbine mixer

2.8 Sterile Petri dish: 90mm in diameter.

2.9 Constant Temperature Water Bath Box: 46℃±1℃.

2.10 Microscope

2.11 Micro pipette and suction head:1.0mL.

2.12 Refractometer

2.13 A special glass slide with a standard measuring chamber.

2.14 Coverslip

2.15 Micrometer: glass slide with standard scale

3 Medium and Reagents

3.1 Sterile saline: see A.1.

3.2 Potato dextrose agar: see A.2.

3.3 Bengal red AGAR: see A.3.

3.4 Phosphate buffer: see A.4.

The first method - Plate counting of mould and yeast

4Test program

The test procedure of mould and yeast is shown in Figure 1

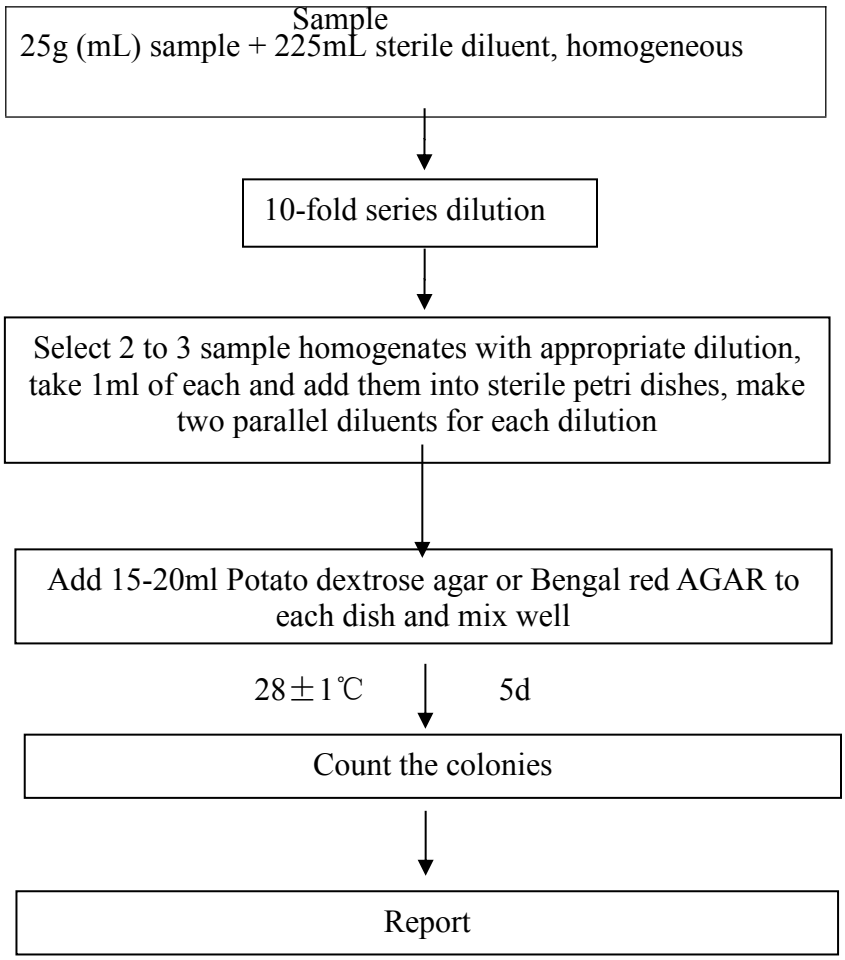

Fig. 1 Test procedure of mould and yeast

5 Operation steps

5.1 Sample dilution

5.1.1 Solid and semi-solid samples: 25g samples were weighed and put into 225mL sterile diluent (distilled water, normal saline and phosphate buffer) ,fully shaken,or flapped for 1min-2min to produce 1:10 sample homogenate

5.1.2 Liquid Samples: 25mL Samples were taken by sterile straw and placed into 225mL sterile diluent (distilled water, normal saline and phosphate buffer) which contained by a flask (A proper number of sterile glass beads were pre-arranged in the flask) or other sterile containers are fully shaken or placed in a mechanical oscillator to shake, fully mix, and make a sample homogenate of 1:10.

5.1.3 Absorb 1mL of 1:10 homogenate with 1mL sterile straw or micropipette, slowly inject it into a sterile test tube containing 9 mL diluent, change another 1mL sterile straw to blow and suck repeatedly, or mix it evently on the rotary mixer to get 1:100 sample homogenate.

5.1.4 Accroding to 5.1.3 operation, 10 times series of diluted sample homogenate was prepared. For each incremental dilution, use a new 1mL sterile straw or suction head.

5.1.5 Accroding to the estimation of the contamination status of samples, select 2~3 sample homogenates with appropriate dilution (liquid samples may include original solution), and 1mL sample homogenates were absorbed into sterile plates during the 10-fold incremental dilution. And each dilution was made into two plates. At the same time, 1mL blank diluent was absorbed and added into two sterile plates as blank control.

5.1.6 Poure 20~25mL potato glucose AGAR or bengal red AGAR medium cooled to 46℃（which can be placed in an constant temperature water bath box with temperature of 46±1℃）into the plate，and turn the plate to mix it well. Set the horizontal mesa until the medium is completely set.

5.2 Cultivate

After AGAR solidification, a positive plate was placed in an incubator at 28±1℃, then observe and record the results until the 5^{th} day.

5.3 Colony counting

5.3.1 Can be visually observed, if necessary, with a magnifying glass or low power lens, record the dilution times and corresponding mould and yeast colonies. Colony count is expressed in colony-forming Units (CFU).

5.3.2 Select a plate with a colony number of 10 CFU to 150 CFU. Mould and yeast were counted accroding to colony morphology. Mould spread and growth over entire plate can be recorded as colony spread.

6 Results and reports

6.1 Results

6.1.1 Calculate the average value of two plate colonies with the same dilution, and then multiply the average value by the corresponding dilution times.

6.1.2 If the number of colonies on two dilution plates is between 10 CFU-150 CFU. Then it shall be calculated in accrodance with the corresponding provisions of GB 4789.2.

6.1.3 If the number of colonies on all dilution plates exceeds 150 CFU, the plate with the highest dilution is counted, and other plates can be recorded as multi-incalculable. The results are calculated by multiplying the average number of colonies by the highest dilution factor.

6.1.4 If all the plate colonies are less than 10 CFU, the average colony number with the lowest dilution should be calculated by multiplying the dilution times.

6.1.5 If there is no colony growth on all dilution plates (including liquid sample stock), the dilution is calculated as less than 1 times the minimum dilution.

6.1.6 If all the dilution plate colonies are not between 10 CFU and 150 CFU, and some of them are less than 10 CFU or more than 150 CFU, then the average colony number closest to 30 CFU or 300 CUF is calculated by multiplying the dilution times.

6.2 Report

6.2.1 The number colonies was revised according to the principle of "rounding off". When the colony number is less than 10, a significant number is used to report. Two digit significant numbers were used to report the number of colonies between 10 and 150.

6.2.2 When the colony number is greater than or equal to 100 CFU, the third digit is corrected by the "rounding" principle. The first two digits are taken, followed by 0 to replace the digit. It can also be expressed as an exponentail form of 10, with two significant digits after the "round off" principle.

6.2.3 If there was colony growth on the blank control, the test result was invalid.

6.2.4 Weight sampling is reported in CFU/g and volume sampling in CFU/ml. Report and separately report mould and/or yeast counts.

The second method direct microscopy for mould counting

7 Operating steps

7.1 Preparation of the sample: An appropriate amount of the sample was diluted with distilled water until the refractive index was 1.3447-1.3460 (the concentration was 7.9%-8.8%)

7.2 Microscope standard vision correction: adjust the standard field of view to the diameter of 1.382mm by 90-125 times of magnification.

7.3 Smear: clean the glass slides, spread the standard liquid evenly in the measuring room with glass rod, and cover the slides for observation.

7.4 Observation: the prepared slides were placed under the microscope standard field of view for observation. Generally, each sample was observed in 50 fields per person. The same sample shall be observed by two persons.

7.5 Results and calculation: in the standard field of vision, if the length of mycelia exceeds 1/6 of the standard field vision (1.382mm) or the total length of three mycelia exceeds 1/6 of the standard field vision (the grid of micrometer), it will be recorded as positive, otherwise it will ber recorded as negative.

7.6 Report: the total number of positive visual fields per 100 visual fields was the percentage of mould visual fields.

Appendix A: Culture Medium and Reagent

A.1 Normal saline (NS)

A.1.1 Compositions

Sodium chloride	8.5g
Distilled water	1000mL

A.1.2 Manufacturing Method

Sodium chloride 8.5 g was weighed and dissolved in 1000 mL distilled water. High-pressure

sterilization at 121℃ for 15 minutes.

A.2 Potato dextrose agar

A.2.1 Compositions

Potatoes (peeled and chopped)	300.0g
Glucose	20.0g
AGAR	20.0g
Chloramphenicol	0.1g
Distilled water	1000mL

A.2.2 Manufacturing Method

Peel and slice the potatoes, add 1000mL distilled water and boil them for 10-20min. Filter them with gauze, add distilled water to 1000ml and add glucose and AGAR, heat them to dissovle, and divide them into separate containers . High-pressure sterilization at 121℃ for 15 minutes.

A.3 Bengal red AGAR

A.3.1 Composition

Peptone	5.0g
Glucose	10.0g
Potassium dihydrogen phosphate	1.0g
Magnesium sulfate (anhydrous)	0.5g
AGAR	20.0g
Rose-bengal	0.033g
Chloramphenicol	0.1g
Distilled water	1000mL

A.3.2 Manufacturing Method

These ingredients are added to distilled water and heated to dissove, dilute it to 1000mL with distilled water,and then separated, sterilized at 121℃ for 15 minutes, and stored away from light for use.

A.4 Phosphate buffer

A.4.1 Component

Potassium dihydrogen phosphate (KH_2PO_4)	34.0g
Distilled water	500mL

A.4.2 Manufacturing Method

Storage solution: 34.0g Potassium dihydrogen phosphate was dissovled in 500mL distilled water, adjust PH to 7.2 by 175mL 1mol/L sodium hydroxide solution, diluted with distilled water to 1000mL, and stored in refrigerator.

Diluent solution: take 1.25mL of the storage liquid, dilute it to 1000mL with distilled water, and placed in a suitable container. High-pressure sterilization at 121℃ for 15 minutes.