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## National Food Safety Standard of P. R. China

GB 4789.15 – 2010

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### Food Microbiological Examination: Enumeration of Yeasts and Molds

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Issued by **Ministry of Health of P. R. China**

## **Foreword**

This national standard will replace GB/T4789.15-2003 Food Microbiological Examination: Enumeration of Yeasts and Molds from the implementation date of this standard.

There have been some changes in this national standard over its previous edition GB/T4789.15-2003 covering the followings:

Modified the scope;

Modified the inspection procedures and operation steps;

Modified the culture medium and reagent;

Modified the annex;

Annex A of this standard is a normative appendix while Annex B of this standard is an informative one.

The issuance status of all the previous editions replaced by this standard were:

—— GB 4789.15 – 1984, GB/T 4789.15 – 1994 and GB/T 4789.15 – 2003.

# National Food Safety Standard of P. R. China

## Food Microbiological Examination: Enumeration of Yeasts and Molds

### 1 Scope

This standard specifies the determination method for yeasts and molds in foods.

This standard is applicable to the determination of yeasts and molds in various kinds of foods.

### 2 Equipment and Material

Besides of the conventional sterilization & cultivation equipments for microbiological lab, following equipments are requested:

- 2.1 Refrigerator: 2°C-5°C
- 2.2 Thermostatic incubator: 28°C ± 1°C
- 2.3 Homogenizer
- 2.4 Constant temperature Oscillator
- 2.5 Microscope: 10 × - 100 ×
- 2.6 Electronic balance: sensitivity accurate to 0.1g
- 2.7 Aseptic conical flask: 500mL, 250mL
- 2.8 Aseptic wide neck flask: 500mL
- 2.9 Aseptic pipette: 1 mL (with calibration 0.01mL), 10mL (with calibration 0.1 mL)
- 2.10 Aseptic flat plate: diameter 90mm
- 2.11 Aseptic test tube, 10mm x 75mm
- 2.12 Aseptic kraft paper bag and plastic bag

### 3 Culture Medium and Reagent

- 3.1 Potato Dextrose Agar (PDA) culture medium; refer to Annex A.1
- 3.2 Rose Bengal Medium: refer to Annex A.2

### 4 Inspection Procedure

Please refer to Fig. 1 for the inspection procedure.

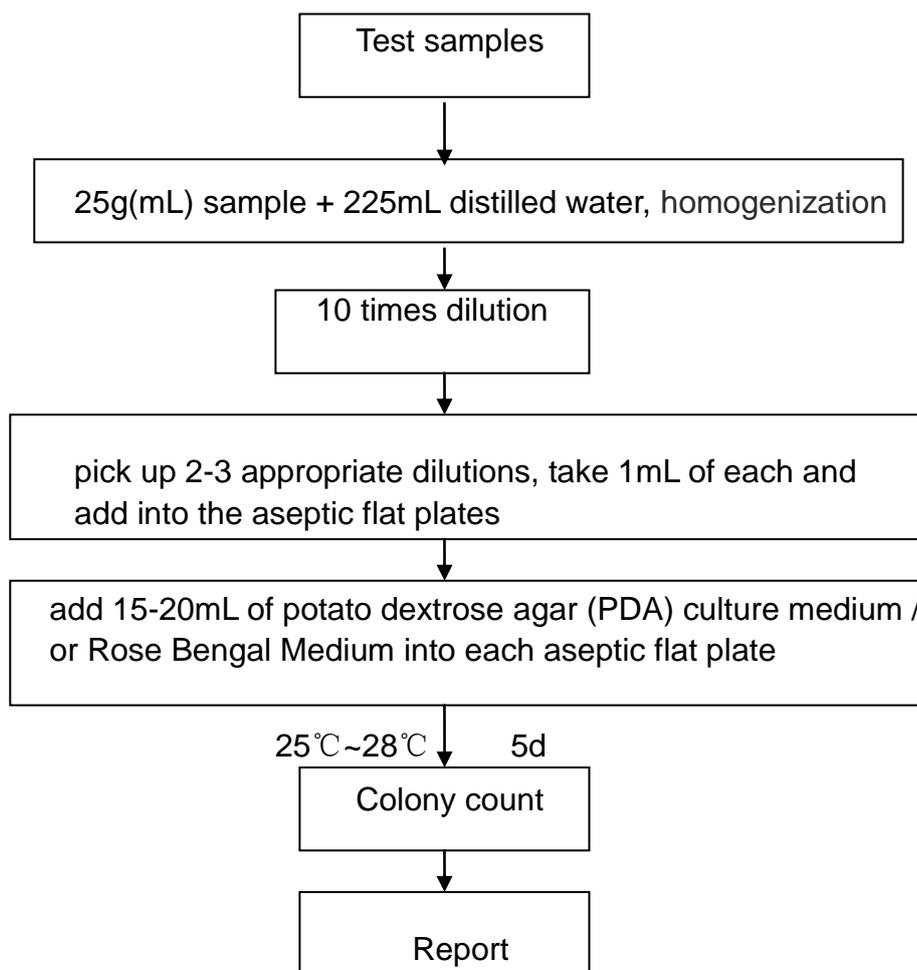


Fig. 1 inspection procedure on yeasts and molds counts

## 5 Operation Steps

### 5.1 Dilution of samples

5.1.1 For solid and semisolid samples: Weigh 25g test sample and put into narrow neck flask which containing 225mL sterile distilled water, shaking to get the 1:10 dilution; Or put the sample into the bagfilter contains 225mL sterile distilled water, then use bagmixer to mix 2 min. to get the 1:10 dilution.

5.1.2 For liquid form sample: use an aseptic pipette to pipet 25mL sample into a narrow neck flask (some aseptic glass beads inside) containing 225mL sterile distilled water, mix well and get a 1:10 sample dilution.

5.1.3 Pipet 1mL of this 1:10 dilution into a test tube contains 9mL of sterile distilled water, use another 1mL aseptic pipette to repeat pipettings until getting a 1:100 dilution.

5.1.4 Repeat step of 5.1.3 to prepare series of 10x dilution. Replace a new aseptic pipette each time when the next dilution is made.

5.1.5 According to the estimation of sample pollution situation, select 2-3 appropriate dilutions (could be incl. the liquid stock solution) and while 10 times dilutions are respectively conducted, pipet 1mL sample solution per every dilution and put solution into a sterilized flat plate. Meanwhile, pipet 1mL sample solution and put solution into a sterilized

flat plate as blank control.

5.16 Cool down the potato dextrose agar (PDA) culture medium or the rose Bengal medium to 46°C (can be kept in the water bath at temp. 46°C  $\pm$ 1°C ), promptly pour 15mL-20mL into the flat plate, rotate the plate and let the medium disperse evenly.

## 5.2 Incubation

After the agar is gelling, put the flat plate upside down and incubate 5 days at 28°C  $\pm$ 1°C. Observe and take record.

## 5.3 Colony count

- Visualizing or use magnifier if necessary, take record of the yeasts and molds corresponding to the different dilution ratio. Expressed as CFU (colony forming units).  
Select the plate with counts between 10CFU – 150 CFU, count the yeasts and molds respectively according to their appearance. Record as TNTC (Too Numerous To Count) if the molds covered the whole plate. 2 Average plate counts should be adopted for colony count.

## 6 Calculation & Report

6.1 Calculate on the average value based on two plate counts, then multiply the value by the corresponding dilution ratio.

6.1.2 If the colony counts from all plates are more than 150 CFU, count the plate with the highest extent of dilution, other plates can be recorded as TNTC (Too Numerous To Count), result can be calculated based on average plate counts multiplying by the highest dilution ratio.

6.1.3 If the colony counts from all plates are less than 10 CFU, then result should be calculated based on average plate counts with the lowest extent of dilution with multiplying by the dilution ratio.

6.1.4 If no colony was found on the plates with each the dilution ratio, then the result will be expressed as less 1 times the lowest dilution ratio; if it comes from the stock solution, then it should be expressed as less 1.

## 6.2 Report

6.2.1 If the colony counts less than 100, should be reported as the rounding rules by “rounding-off” and get the 2 significant digits as result.

6.2.2 If the colony counts more than 100(incl. 100), the first 3 digits should be “rounding-off” and the result will be expressed as fist 2 digits with 0 replace the last digit; Another way can be also expressed as exponential of 10, under the same principal of “rounding-off” and use 2 significant digits.

6.2.3 CFU/mL should be regarded as the unit by weigh sampling in the report, while CFU/g should be regarded as the unit by volume sampling. Colony counts on molds and/or yeasts can be applicable.

**Appendix A**  
**(Normative Appendix)**  
**Culture Medium and Reagent**

## A.1 Potato Dextrose Agar (PDA) culture medium

## A.1.1 Ingredients

Potatos (peeled, diced)	300g
Dextrose	20.0g
Agar	20.0g
Chloromycetin	0.1 g
Distilled water	1000 mL

## A.1.2 Preparation

Peel the potatoes, put in 1000mL distilled water, boiling for 10-20 min. After sieving by gauze, refill distilled water to 1000 mL. Add in dextrose and agar, melt down then filling. 121°C sterilize for 20 min. Use small amount of ethanol to dissolve Chloromycetin and put into the culture medium before pouring into the flat plates.

## A.2 Rose Bengal Medium

## A.1.1 Ingredients

Peptone	5.0 g
Dextrose	10.0g
Monopotassium phosphate	1.0 g
Magnesium sulfate (anhydrous)	0.5 g
Agar	20.0g
Rose Bengal	0.033g
Chloromycetin	0.1 g
Distilled water	1000 mL

## A.1.2 Preparation

Mix all above ingredients and put into distilled water, heat up until melt down, refill distilled water to 1000 mL, filling, 121°C sterilize for 20 min. Use small amount of ethanol to dissolve Chloromycetin and put into the culture medium before pouring into the flat plates.

**Appendix B**  
**(Informative Appendix)**

**Direct Microscopic Determination of Molds**

The commonly adopted method is Howard's method. This method is applicable to tinned tomato ketchup.

**B.1 Equipment and Material**

B.1.1 Refractometer

B.1.2 Microscope

B.1.3 Howard's counting slide: special slide with standard determination chamber

B.1.4 Cover glass

B.1.5 Micrometer: slide with standard scale

**B.2 Operation Steps**

B.2.1 Preparation of test sample: Take a Quantitation of the test sample, add distilled water to dilute it till the refractive index is 1.3447 ~ 1.3460 (which means that the concentration is 7.9% ~ 8.8%), the dilution now is ready for use.

B.2.2 Adjustment of the standard visual field of microscope: Adjust the standard visual field of the microscope according to the magnification of 90-125 times and make the diameter as 1.382 mm.

B.2.3 Smear: clean the Howard's counting slide, spread the prepared standard solution with a glass rod evenly in the determination chamber for observation.

B.2.4 Observation: Place the prepared slide under the standard visual field of the microscope for molds observation. Usually 50 visual fields should be observed for each test sample and one test sample should be observed by two persons.

B.2.5 Result and calculation: In standard visual field, when it is discovered that the length of the hypha exceeds 1/6 of the standard visual field (1.382mm) or the total length, or three hypha exceeds 1/6 of the standard visual field (one grid of the micrometer), it is positive (+); otherwise, it is negative (-). Based on 100 visual fields, the number of visual fields, in which the existence of mold hypha is discovered, is the visual field percentage of molds.

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