

# NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

# 中华人民共和国国家标准

GB 5750.12–2006 Partly Replaced GB 5750-1985

# Standard Examination Methods for Drinking Water — Microbiological Parameters 生活饮用水标准检验方法 微生物指标

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#### **Foreword**

GB/T 5750 Standard examination methods for drinking water divided into following parts:

- General principles;
- Collection and preservation of water samples;
- Water analysis quality control;
- Organoleptic and physical parameters;
- Nonmetal parameters;
- Metal parameters;
- Aggregate organic parameters;
- Organic parameters;
- Pesticides parameters;
- Disinfection by-products parameters;
- Disinfectants parameter;
- Microbiological parameters;
- Radiological parameters.

This Standard will replace Total bacterial count and total coliform in second clause those specified in GB/T 5750-1985 Standard examination methods for drinking water.

Comparison with GB/T 5750-1985, main changes of this Standard are as follows:

- To adjust the structural based on GB/T 1.1-2000 *Directives for Standardization-Part 1:Rules for the structure and drafting of standards*;
- To add seven examination methods of four item indexes to drinking water such as thermotoletant coliform bacteria, Escherichia coli, Giardia lamblia and Cryptosporidiuin.

This Standard is proposed and under the jurisdiction of Ministry of Health of the People's Republic of China.

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This Standard was first issued on August, 1985, this is first revision.

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#### Standard examination methods for drinking water—

#### Microbiological parameters

#### 1. Standard Plate-count Bacteria

#### 1.1 Plate Counting Method

#### 1.1.1 Scope

This standard provides to determine total number of bacterial colony in drinking water and its source by plate counting method.

This method applies to determination of bacterial colony in drinking water and its source.

#### 1.1.2 Terms and Definitions

The following terms and definitions apply to this standard.

1.1.2.1

#### Standard plate-count bacteria

Standard plate-count bacteria in 1 mL water sample after 48 hours of cultivation on nutrient agar under aerobic condition of 37 °C.

#### 1.1.3 **Medium and Reagent**

#### 1.1.3.1 **Nutrient Agar**

#### 1.1.3.1.1 Ingredients:

| A | peptone         | 10g     |
|---|-----------------|---------|
| В | beef extract    | 3g      |
| C | sodium Chloride | 5g      |
| D | agar            | 10g~20g |
| E | distilled water | 1000mL  |

1.1.3.1.2 Method: Mix the ingredients mentioned above and heat them to dissolve, adjust pH to 7.4~7.6, and put them separately into glass container (If the agar contains more impurities, it should be filtered firstly), after 20 minutes of sterilization of 103,43 kPa (121°C,15lb) store them in dark cold place as preparation.

#### 1.1.4 Instrument

- 1.1.4.1 Autoclave sterilizer.
- 1.1.4.2 Oven.
- 1.1.4.3 Incubator  $36^{\circ}C\pm 1^{\circ}C$ .
- 1.1.4.4 Electric furnace.
- 1.1.4.5 Balance.

- 1.1.4.6 Refrigerator.
- 1.1.4.7 Magnifier or bacterial colony counter.
- 1.1.4.8 pH meter or accurate pH test paper.
- 1.1.4.9 Sterilized tube, plate (diameter 9mm), calibrated pipette, sampling bottle and so on.

#### 1.1.5 **Inspection Procedure**

#### 1.1.5.1 **Drinking Water**

- 1.1.5.1.1 Absorb 1mL water sample mixed totally using sterilized tube by sterile method and then inject the water sample into sterilized plate, also pour about 15mL nutrient agar cultivating medium that is melted and cooled to 45°C, spin and shake the plate at once to make water sample mix with cultivating medium completely. Do a paralleled inoculation for each examination and at the same time pour nutrient agar cultivating medium only into another plate as blank contrast.
- 1.1.5.1.2 After cooling and solidification, turn the plate and make the bottom upward and put it into cultivating box of  $36^{\circ}\text{C}\pm1^{\circ}\text{C}$  for 48 hours, and then count the bacterial colonies, namely the total number of bacterial colony in 1 mL water sample.

#### 1.1.5.2 Source Water

- 1.1.5.2.1 Absorb 1 mL water sample mixed completely by sterile method and inject into tube filled with 9 mL sterilized normal saline, then mix them into diluent of 1:10.
- 1.1.5.2.2 Absorb 1 mL diluent of 1:10 and inject into tube filled with 9 mL sterilized normal saline and mix them into diluent of 1:100. And also make diluent of 1:1000, 1:10000 by the same method as preparation. You must change a sterilized tube of 1 mL for each diluent.
- 1.1.5.2.3 Absorb undiluted water sample by sterilized tube and 2~3 1mL water sample diluted properly, and inject them separately into sterilized plates. The following operation is same as examination steps of drinking water.

#### 1.1.6 Colony Count and Reporting Methods

When counting bacterial colony in plate, we can observe directly by eyes and use magnifier when necessary in case of omitting. After recorded bacterial colony in every plate, average colony number with dilution should be made for applying to next calculation. The rather big slice of bacterial colony in one plate should not for be adopted for average dilution, while plate without big slice is appropriate for average colony number of this dilution. If the slices of bacterial colony are less than half of plate and the bacterial colony in another half is well-distributed, this well-distributed half of plate can be counted and multiply 2 to represent bacterial colonies in whole plate, and then make average bacterial colony in this dilution.

#### 1.1.7 Select to Different Dilution and Reporting Methods

1.1.7.1 Firstly it should be calculated by selecting average bacterial colony between 30 and 300. If average



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