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National Food Safety Standard – Food Microbiological Examination – Commercial Sterilization

食品安全国家标准 食品微生物学检验 商业无菌检验

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National Food Safety Standard – Food Microbiological Examination – Commercial Sterilization

1 Scope

This Standard specifies food commercial sterility inspection procedures, inspection procedures, result determination and report requirements.

This Standard applies to the inspection of food commercial sterility.

2 Terms and Definitions

2.1 Commercial sterility

A state in which food has been moderately heat sterilized and does not contain pathogenic microorganisms or non-pathogenic microorganisms that can reproduce in it at normal temperatures.

2.2 Low-acid food

Any food with a balanced pH greater than 4.6 and a water activity greater than 0.85 after sterilization.

2.3 Acidic foods

Foods that have not been acidified, and after sterilization, the balanced pH of the food itself or the soup is equal to or less than 4.6, and the water activity is greater than 0.85. Tomato products with a pH less than 4.7 are acidic foods.

2.4 Acidified foods

Foods whose water activity is greater than 0.85 and whose balance pH is equal to or less than 4.6 after adding acidity regulators or acidifying the food through other acidification methods.

3 Equipment and Materials

In addition to the routine sterilization and culture materials and equipment of the microbiology laboratory, other equipment is as follows.

3.1 Refrigerator: 2℃~5℃;

3.2 Constant temperature incubator: 30℃±1℃, 36℃±1℃, 55℃±1℃;

3.3 Constant temperature culture chamber: 30℃±2℃, 36℃±2℃, 55℃±2℃;

3.4 Constant temperature water bath: 55℃±1℃;

3.5 Homogenizer and sterile homogenization bag, homogenization cup or mortar;

3.6 Potentiometric pH meter: Accuracy is 0.01pH;

3.7 Microscope objective lens: 10×~100×;

3.8 Can punch or container opener;

3.9 Anaerobic incubator (can).

4 Culture Media and Reagents

4.1 Culture medium: See Appendix A.

4.2 Crystal violet staining solution: See A.8 in Appendix A.

4.3 Gram staining solution: See A.9 in Appendix A.

4.4 Sterile physiological saline: See A.10 in Appendix A.

4.5 Xylene.

4.6 Ethanol solution containing 4% iodine: 4g of iodine is dissolved in 100mL of 70% ethanol solution.

4.7 75% ethanol solution: Measure 75mL of absolute ethanol and 25mL of water respectively, mix well and set aside.

4.8 70% ethanol solution: Measure 70mL of absolute ethanol and 30mL of water respectively, mix well and set aside.

5 Commercial Sterility Inspection in Food Circulation Field

5.1 Inspection procedures

The commercial sterility inspection procedures in the food circulation field are shown in Figure 1.

After taking the sample, record the product name and number, and mark the surface of the sample packaging. Make sure that the sample has a normal appearance and no obvious damage, rust (only for metal containers), leakage, bulging cans (bags, bottles, cups, etc.), and the like abnormal situation.

5.2.2 Heat preservation

Take 1 sample from each batch and store it in a refrigerator at 2℃~5℃ as a control, and keep the remaining samples at 36℃±1℃ for 10 d. During the heat preservation process, regular inspections shall be made every day. If there is any expansion or leakage in the can (bag, bottle, cup, etc.), it shall be taken out immediately; opened, inspected and recorded according to 5.2.3.

5.2.3 Opening food containers

5.2.3.1 After cooling all heat-preserved sample to normal temperature; start the inspection according to sterile operation.

5.2.3.2 If there is expansion or leakage in the cans (bags, bottles, cups, etc.) during the heat preservation process, they shall be removed immediately. The severely expanded samples shall be placed in a refrigerator at 2℃~5℃ for several hours before opening the food container for inspection.

5.2.3.3 After the sample to be tested is carried out heat preservation, if necessary, the outer surface of the sample to be tested can be cleaned with warm water or detergent. After rinsing with water, wipe it with a sterile towel (cloth or paper) or sterile cotton (containing 75% ethanol solution) to dry. Soak in ethanol solution containing 4% iodine (or 75% ethanol solution) to disinfect the outer surface for 30 min; then dry it with a sterile towel and turn it on; or ignite it in a closed cover until all the remaining iodine ethanol solution on the surface is burned and then turn it on (expanded samples and samples in containers with flammable packaging materials cannot be burned).

5.2.3.4 Test samples shall be opened according to sterile operation requirements. Samples with soup shall be shaken properly before opening. For metal container samples, use a sterile can opener or can puncher to open an appropriately sized opening on the smooth surface of the sterilized can or directly pull the ring to open it. Do not damage the curling structure when opening the can. Before each can opening, the can opener shall be ensured to be in a sterile state to prevent cross-contamination. For soft-packaged samples, sterilized scissors can be used to open them; and the interface must not be damaged.

NOTE: Samples from severely bulging cans (bags, bottles, cups, etc.) may explode and emit toxic substances. Preventive measures can be taken such as covering the sample with a sterile towel or using a sterile funnel to tip it upside down on the sample to prevent such dangers from occurring.

5.2.4 Retention samples

After opening, use a sterile straw or other appropriate tools to take out at least 30mL (g) of the

contents into a sterilized container from a sterile operation; and store it in a refrigerator at 2°C ~ 5°C. It can be used for further testing when necessary. The sample can be discarded after reaching the test conclusion.

5.2.5 Sensory inspection

In an inspection chamber with sufficient light and clean air and no odor, pour the sample contents into a white enamel plate or glass container (suitable for liquid samples); and observe and smell the structure, shape, color, and smell of the product. Samples containing solid matter shall check the product properties according to the food; identify whether the food has signs of corrosion and deterioration; and observe the conditions inside the packaging container and record them.

5.2.6 pH measurement and result analysis

5.2.6.1 Determination

Canned food shall be tested according to the method specified in GB 5009.237. Follow the same instructions for other foods.

5.2.6.2 Analysis of result

Compare whether there is any significant difference with the control sample stored under refrigeration in the same batch. A pH difference of 0.5 or more is considered a significant difference.

5.2.7 Stained smear microscopy

5.2.7.1 Smear

Take the sample contents for smear. Samples with soup can use an inoculation loop to pick up the soup and apply it on a glass slide. Solid foods can be smeared directly or diluted with a small amount of sterile physiological saline and smeared; and then fixed with a flame after drying. After the greasy food smear is naturally dried and flame-fixed, it is washed with a degreasing agent such as xylene and dried naturally.

5.2.7.2 Staining microscopy

Single-stain the smear in 5.2.7.1 with crystal violet staining solution; dry it and inspect it under the microscope. Observe at least 5 fields of view and record the morphological characteristics of the bacteria and the number of bacteria in each field of view. Compared with the refrigerated storage control samples of the same batch, determine whether there is obvious microbial proliferation. An increase of a hundred times or more in the number of bacteria is regarded as significant proliferation.

5.3 Result judgment and report

5°C. It can be used for further testing when necessary. The sample can be discarded after reaching the test conclusion. The opened sample container can be properly stored for future container inspection.

6.2.5 Sensory inspection

The retention sample shall be carried out according to the procedures specified in 5.2.5.

6.2.6 pH measurement and result analysis

Manufacturers shall establish the normal pH control range for this type of product based on the product properties. The pH shall be measured in accordance with GB 5009.237 or relevant standards. If the pH value exceeds the normal control range, staining microscopy shall be performed.

6.2.7 Stained smear microscopy

6.2.7.1 Smear

Canned samples that are considered suspicious by sensory or pH test results, as well as those whose pH response is insensitive during spoilage (such as meat, poultry, fish, etc.) shall be subjected to smear staining and microscopic examination.

Take the sample contents for smear. Samples with soup can use an inoculation loop to pick up the soup and apply it on a glass slide. Solid foods can be smeared directly or diluted with a small amount of sterile physiological saline and smeared; and then fixed with a flame after drying. After the greasy food smear is naturally dried and flame-fixed, it is washed with a degreasing agent such as xylene and dried naturally.

6.2.7.2 Staining microscopy

Single-stain the smear in 6.2.7.1 with crystal violet staining solution; dry it and inspect it under the microscope. Observe at least 5 fields of view and record the morphological characteristics of the bacteria and the number of bacteria in each field of view.

Manufacturers can establish criteria for judging the obvious proliferation of microorganisms in this type of products based on product characteristics. Compared with the judgment criteria or normal samples from the same batch [such as unexpanded cans (bags, bottles, cups, etc.), samples with no sensory abnormalities], judge whether there is obvious microbial proliferation.

6.2.8 Inoculation and culture

If the expanded cans (bags, bottles, cups, etc.), leakage or opening during the heat preservation period and find abnormal pH, sensory quality, or spoilage, and further microscopic examination reveals an abnormal number of bacteria in the sample, it shall be inoculated and cultured with microorganisms, as well as carry out anomaly analysis in accordance with Appendix B.

Appendix A

Culture Media and Reagents

A.1 Bromocresol purple glucose broth

A.1.1 Ingredients

Peptone: 10.0g;

Beef extract: 3.0g;

Glucose: 10.0g;

Sodium chloride: 5.0g;

Bromocresol purple: 0.04g (or 2.0mL of 1.6% ethanol solution);

Distilled water: 1000.0mL.

A.1.2 Preparation method

Heat, stir and dissolve all ingredients except bromocresol purple; correct the pH to 7.0±0.2; add bromocresol purple. And distribute into test tubes with small inverted tubes; 10 mL per tube; and autoclave at 121°C for 10 min.

A.2 Cooked meat culture medium

A.2.1 Ingredients

Beef infusion: 1000.0mL;

Peptone: 30.0g;

Yeast paste: 5.0g;

Glucose: 3.0g;

Sodium dihydrogen phosphate: 5.0g;

Soluble starch: 2.0g;

Minced meat residue: Appropriate amount.

A.2.2 Preparation method

A.2.2.1 Weigh 500g of fresh ground beef with the fat and fascia removed; add 1000 mL of distilled water and 25.0 mL of 1 mol/L sodium hydroxide solution; stir and boil for 15 min; fully cool. Remove surface fat; clarify; filter; and add water to make up to 1000mL, which is the beef infusion. Add various ingredients in A.2.1 except minced meat residue, and correct the pH to 7.8±0.2.

A.2.2.2 Wash the minced meat residue and dry it until semi-dry. Divide into 15mm×150mm test tubes, 2cm~3cm high. Add 0.1g~0.2g of reduced iron powder or a little iron filing to each tube. Dispense the liquid culture medium prepared in A.2.2.1 into each tube so that it exceeds the surface of the meat residue by about 1cm. Cover it with 0.3cm~0.4cm of melted petroleum jelly or liquid paraffin. Autoclave at 121°C for 15 min.

A.3 Nutrient agar

A.3.1 Ingredients

Peptone: 10.0g;

Beef paste: 3.0g;

Sodium chloride: 5.0g;

Agar: 15.0g~20.0g;

Distilled water: 1000.0mL.

A.3.2 Preparation method

Dissolve all ingredients except agar in distilled water; add about 2 mL of 15% sodium hydroxide solution; and calibrate the pH to 7.2~7.4. Add agar, heat and boil to dissolve the agar. Aliquot into flasks or 13mm×130mm test tubes and autoclave at 121°C for 15 min.

A.4 Acidic broth

A.4.1 Ingredients

Polyvalent peptone: 5.0g;

Yeast extract: 5.0g;

Glucose: 5.0g;

Potassium dihydrogen phosphate: 5.0g;

Distilled water: 1000.0mL.

A.4.2 Preparation method

Soluble starch: 10.0g;

Plasma casein: 2.0g;

Sodium chloride: 5.0g;

Sodium nitrate: 2.0g;

Gelatin: 20.0g;

Agar: 15.0g;

Distilled water: 1000.0mL.

A.7.2 Preparation method

Mix ingredients in distilled water. Calibrate the pH to 7.3±0.2 and sterilize at 121°C for 15 min.

A.8 Crystal violet staining solution

A.8.1 Ingredients

Crystal violet: 1.0g;

95% ethanol: 20.0mL;

1% ammonium oxalate aqueous solution: 80.0mL.

A.8.2 Preparation method

Completely dissolve 1.0g of crystal violet in 95% ethanol; and then mix with 1% ammonium oxalate solution.

A.8.3 Staining method

Fix the smear on the flame of an alcohol lamp; titrate crystal violet dye; dye for 1 min; and wash with water.

A.9 Gram stain solution

A.9.1 Crystal violet staining solution

The same as A.8.

A.9.2 Gram's iodine solution

A.9.2.1 Ingredient

Iodine: 1.0g;

Potassium iodide: 2.0g;

Distilled water: 300.0mL.

A.9.2.2 Preparation method

Mix 1.0g of iodine and 2.0g of potassium iodide first; add a little distilled water and shake thoroughly. After complete dissolution, add distilled water to 300mL.

A.9.3 Safranin counterstain solution

A.9.3.1 Ingredients

Safranin: 0.25g;

95% ethanol: 10.0mL;

Distilled water: 90.0mL.

A.9.3.2 Preparation method

Dissolve 0.25g of Safranin in ethanol and then dilute with distilled water.

A.9.4 Staining method

A.9.4.1 Fix the smear on the flame; titrate crystal violet staining solution; stain for 1 min; and wash with water.

A.9.4.2 Titrate Gram's iodine solution; let it act for 1 min; and wash with water.

A.9.4.3 Titrate 95% ethanol for decolorization for 15s~30s until the staining solution is washed away. Do not decolorize excessively and wash with water.

A.9.4.4 Titrate counterstaining solution; counterstain for 1 min; wash with water; wait to dry; and inspect under microscope.

A.10 Stroke physiological saline solution

A.10.1 Ingredients

Sodium chloride: 8.5g;

Distilled water :1000.0mL.

A.10.2 Preparation method

Weigh 8.5g of sodium chloride and dissolve in 1000mL of distilled water; and autoclave at 121°C for 15 min.

B.2.9 Analysis of result

B.2.9.1 If no microbial growth is found in the sample of the expanded can, the expansion may be caused by the reaction between the contents and the metal container to produce hydrogen gas. The amount of produced hydrogen varies with the duration of storage and storage conditions. Overfilling may also cause slight expansion, which can be determined by weighing.

A mixed bacterial phase with a large number of bacteria was seen in the direct smear, but did not grow after culture, indicating spoilage that occurred before sterilization. As a result of bacterial growth before the container is sealed, the product's pH, odor, and tissue morphology appear abnormal.

B.2.9.2 When the sealing of food containers is good, if only Bacillus grows under the culture conditions at 36°C and their heat resistance is no higher than that of Clostridium botulinum (C.botulinum), it indicates that sterilization is carried out during the production process insufficiently.

B.2.9.3 The presence of mixed colonies of bacilli, cocci, and fungi in culture indicates that the food container is leaking. It may also be caused by insufficient sterilization, but in this case the expansion rate of the same batch of products shall be very high.

B.2.9.4 Incubate in bromocresol purple glucose broth at 36°C or 55°C and observe acid production and gas production. If acid production occurs, it indicates the presence of mesophilic microorganisms, such as mesophilic acid-resistant Bacillus or thermophilic microorganisms, such as the growth of B. stearothermophilus.

Bacteria grew and produced gas on the cooked meat culture medium at 55°C, emitting a putrid smell, indicating that the sample spoilage was caused by thermophilic anaerobic Clostridium.

If it grows on cooked meat culture medium at 36°C and produces gas with a putrid smell, and spores can be seen under the microscope, indicating that the spoilage may be caused by Clostridium botulinum (C.botulinum), Clostridium sporogenes (C.sporogenes) or Clostridium perfringens (C. perfringens). Further botulinum toxin testing can be performed if necessary.

B.2.9.5 The spoilage of acid foods is usually caused by non-spore-forming lactobacilli and yeasts. Generally, deterioration caused by Bacillus shall not occur when the pH is lower than 4.6, but deteriorated tomato pastes or tomato juice cans do not swell; but they have a rancid smell, with or without a decrease in pH; generally, due to aerobic Bacillus species.

B.2.9.6 Some cans may contain spores of thermophilic bacteria due to non-standard sterilization intensity or cooling process, which do not grow under normal storage conditions; but when the product is stored at a higher temperature (50℃~55℃), thermophilic bacteria shall grow and cause spoilage. Thermophilic and acidophilic Bacillus and Bacillus stearothermophilus cause spoilage in acidic and low-acidic foods respectively but do not cause packaging container swelling. Incubation at 55°C shall not cause changes in the appearance of the packaging

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