National Standards of the People's Republic of China

GB 4789.26—xxxx

National Food Safety Standard

Microbiological Test of Food

Commercial Sterilization Test of Canned Food

(Exposure Draft)

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Preface

This National Food Safety Standard replaces GB/T 4789.26-2003 *Microbiological Test of Food Hygiene - Commercial Sterilization Test of Canned Food*. Compared with GB/T 4789.26-2003, this Standard has mainly undergone the following modifications:

- The Chinese and English names of the Standard have been altered;
- Scope of application has been changed;
- Normative referential documenta have been deleted;
- Terms and definitions have been modified;
- Equipment and materials have been altered;
- Culture medium and reagent have been changed;
- Figures of inspection procedures have been added;
- Inspection procedures have been modified;
- Result determination has been modified;
- Normative appendix A and informative appendix B have been amended.

National Food Safety Standards
Microbiological Test of Food – Commercial Sterilization Test of Canned Food

1. Scope

The standard specifies the basic requirements, operation procedures, and determination of results for commercial sterilization test of canned food.
The standard is applicable to all canned food that has been packaged with various airtight containers.The canned food has undergone proper commercial sterilization by means of heat sterilization so that it can be kept for a long time under room temperature.

2. Terms and Definitions

2.1 Commercial Sterilization of Canned Food

Commercial sterilization means that canned food is free of microorganisms that can reproduce under room temperature and pathogenic microorganism after undergoing proper heat sterilization.

2.2 Hermetical Seal

Hermetical seal means the sealed food container is under the state of being able to prevent microorganisms from getting into it.

2.3 Swell

The package swells because of positive pressure generated by gases produced by microorganisms' activities in the canned food or by other physical and chemical reasons.

2.4 Leakage

The sealing of the canned food has defects, or due to the damage caused by external force, or holes appearing on the food container because of corrosion.

3. Equipment and Materials

Besides the conventional sterilization and culture equipment used in microbiological labs, other following equipments and materials are as follows:

3.1 Refrigerator：2℃～5℃.

3.2 Constant temperature culture medium：36℃ ± 1℃.

3.3 Homogenizer and sterile homogenizing bag, homogenizing cup or a mortar.

3.4 Potentiometric pH meter whose accuracy should be within 0.05 pH unit of the known buffer solution.

3.5 Microscope：10 × ～ 100 ×.

3.6 A bacti-dise can opener used in bacteriology inspection and a can puncher (The best can opener for bacteriology inspection of canned food is composed of a metal rod and a triangular blade, the end of the metal rod is equipped with a punching device, and the triangular blade, which can slide, is arranged on the rod and is fixed by a bolt. Compared with other types of can openers, this can opener won't easily damage the hemming structure of the can and therefore, won't affect leakage check of the canned food.)

3.7 Electronic balance or a bench balance.

3.8 Clean bench or Class 100 clean operating lab.

4 Culture Medium and Reagent

4.1 Sterile normal saline: see A.1 in Appendix A.

4.2 Crystal violet staining solution: see A.2 in Appendix A.

4.3 Dimethylbenzene.

4.4 Ethanol solution with 4% iodine: 4% iodine dissolves in 70% ethyl alcohol.

5 Test procedures

Procedures of commercial sterilization test of canned food are demonstrated in Figure 1.

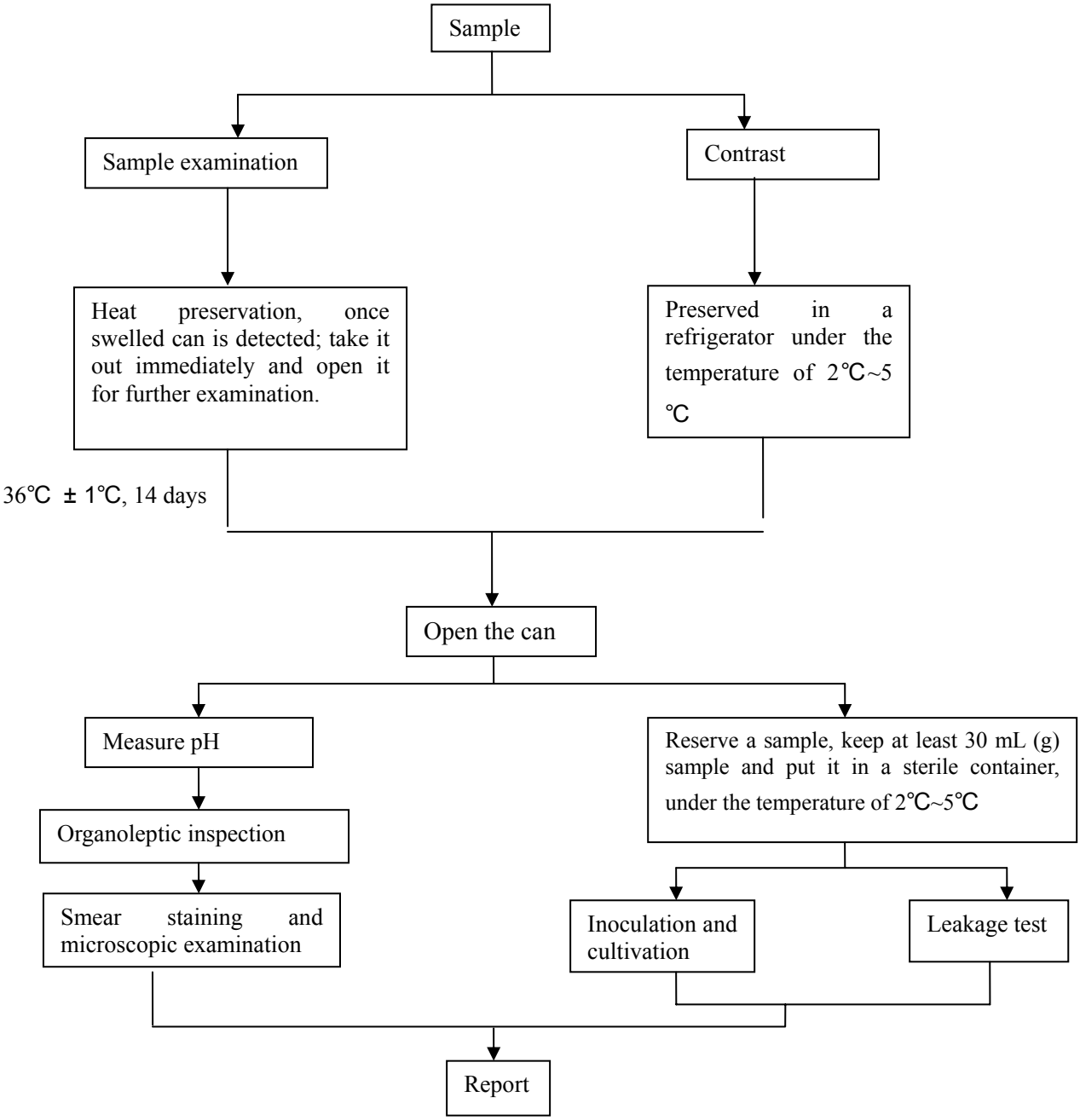

Figure1. Procedures of Commercial Sterilization Test of Canned Food

6 Operation Procedures

6.1 Preparations of the canned food

Remove the label on the can, mark the can with a water-proof oil marking pen, and record such information as the container, number, properties of the product, status of leakage, and whether there are small holes or corrosion, indentation, swell and other abnormal conditions.

6.2 Weighing

Use the electronic balance or bench balance to weigh the canned food; the weight should be as accurate as 1 g for canned food of 1 kg or less, as accurate as 2 g for canned food of more than 1 kg, and as accurate as 10 g for canned food of more than 10 kg, and write down the records.

6.3 Heat preservation

6.3.1 Take one can from each batch and preserve it in a refrigerator under the temperature of 2□ ~ 5□ as the control group. The rest cans shall be preserved under the temperature of 36□ ± 1□ for 14 days. During the process of heat preservation, check the cans every day. If swell or leakage happens, take out the relevant cans immediately and open them for inspection.

6.3.2 After heat preservation, weigh the cans again and write down the records. Compare the newly obtained weight with the weight obtained before heat preservation. If the cans weigh less than its original weight, leakage must have happened. Store all the cans under room temperature until opening them for inspection.

6.4 Open the can

6.4.1 If there are swelled cans, preserve these cans in the refrigerator under the temperature of 2℃ ~ 5℃ for several hours before opening them.

6.4.2 Clean the smooth surface of the sample can that is to be tested with cold water and detergent, wash the can with tap water, and then dry the can with a sterile towel. Sterilize the smooth surface of the can by immersing the smooth surface in ethanol solution with 4% iodine for 15 minutes, and then dry the can with a sterile towel. Ignite the can under an enclosed hood until all the residual iodine ethanol solution on the surface of the can is burned up. Don't ignite any swelled can. Sterilize the smooth surface of the swell can by immersing the smooth surface in ethanol solution with 4% iodine for 30 minutes, and then dry the can with a sterile towel.

6.4.3 Open the can on the clean bench or in the Class 100 clean operating lab. Shake the can appropriately before opening it if there is soup in the can. Make an opening of appropriate size on the sterilized smooth surface of the can with a sterilized can opener. Do not damage the hemming structure while opening the can. Use a different can opener for each can, and do not misuse them. If the sample is flexible packaging product, open the package with a pair of sterilized scissors, and do not damage the interface. Smell the product above the opening immediately, and keep records.

Note: Severely swelled cans might explode and eject toxic substances. Therefore, it is necessary to take some precautionary measures to prevent such danger from happening. For example, put a sterilized towel on the cover of the swelled can or put a sterilized funnel upside down on the can.

6.5 Reserve samples

After opening the can, take out at least 30 mL (g) contents from the can with a sterilized suction tube or other proper tools by sterile operation and reserve the contents in a sterilized container in a refrigerator under the temperature of 2℃ ~ 5℃. The reserved sample can be used for further test when necessary, and can be abandoned after conclusions are drawn for the test of this batch of canned food. The opened canned food can be preserved appropriately for some time in case it is used for container inspection in the future.

6.6 pH measurement

6.6.1 Sample treatment

6.6.1.1 Mix the liquid products for future use. As for products with separated solids and liquids, take out the well mixed liquids for future use.

6.6.1.2 In terms of stiff and half-still products and products from which liquids can barely be separated (such as syrup, jam, jelly, etc.), take a portion of the product and grind it in a homogenizer or mortar. If the sample is still too stiff after being grinded, add in the same amount of sterile distilled water and mix them for future use.

6.6.2 Measure

Insert the electrode into the sample liquid being tested, and adjust the temperature corrector of the pH meter to the temperature of the liquid being tested. If the meter does not have a temperature correction system, adjust the

temperature of the liquid being test to the range of 20℃ ± 2℃, and follow the procedures appropriate for the adopted pH meter. When the indication is stable, read the pH directly from the scale of the meter, and the reading should be as accurate as 0.05 pH unit.

The same sample should be tested at least twice. The difference between the two test results should not exceed 0.1 pH unit. The arithmetic mean value of the two test results is determined as the final result, which should be as accurate as 0.05 pH unit in the report.

6.6.3 Analyzing the result

Compare the result with that of the can preserved in the refrigerator as the control group to see whether there is significant difference. If the pH values differ by more than 0.5, then the difference should be regarded as a significant one.

6.7 Organoleptic Inspection

In a test room with adequate light and clean air free of peculiar odor, pour the contents of the can into a white enamel plate, observe the structure, form and color of the product, smell it, and press it with a piece of tableware or a finger wearing fingerstall, with the purpose of examining the properties of the product and identifying whether the food has gone bad. Meanwhile, observe the can externally and internally, and write down the records.

6.8 Smear staining and microscopic examination

6.8.1 Smear

Apply the sample of the canned food to a smear. If there is liquid in the sample of the canned food, apply the liquid to a glass slide with an inoculating loop. Solid food can directly smear or can be diluted by a small amount of normal saline before smearing, and the solid food has to be fixed with the aid of flame after getting dry. Use dimethylbenzene to wash grease food smear which has been fixed with the help of flame after air drying, and then the grease food shall be dried naturally.

6.8.2 Staining and microscopic examination

Stain these smears with crystal violet staining solution. When they dry up, examine them with a microscope, observe at least five fields, and record the morphological characteristics of the bacteria and the bacterial count in each field. Compare the result with that of the control group, i.e. the canned food preserved in the refrigerator, and determine whether the microorganisms have proliferated obviously. If the bacterial count has increased by one hundred times or more, obvious proliferation has occurred.

7 Result Determination

No leakage is detected after heat preservation of the sample; after the heat preservation, the can is opened for organoleptic inspection, pH value measurement, and smear microscopic examination. If the results show that there is no microorganism proliferation, then the sample shall be reported as commercial sterilization.

Leakage is deteted after heat preservation of the sample; after the heat preservation, the can is opened for organoleptic inspection, pH value measurement, and smear microscopic examination. If the results prove that there is microorganism proliferation, then the sample shall be reported as non-commercial sterilization.

Appendix A

Culture Medium and Reagent

A.1 **Sterile Normal Saline**

A.1.1 **Ingredients**

Sodium chloride	8.5 g
Distilled water	1,000.0 mL

A.1.2 **Preparation**

Weigh 8.5 g sodium chloride and add it into 1000 mL distilled water, and then the solution shall undergone high-pressure sterilization under the temperature of 121□ for 15 minutes.

A.2 **Crystal violet staining solution**

A.2.1 **Ingredients**

Crystal violet	1.0 g
95% ethanol	20.0 mL
1% Ammonium oxalate aqueous solution	80.0 mL

A.2.2 **Preparation**

Let the crystal violet totally dissolve in ethanol, and then mix the ethanol with the ammonium oxalate solution.

A.2.3 **Staining method**

Fix the smears above the flame of an alcohol burner, add in droplets of crystal violet staining solution to stain the smears for 1 minute, and then wash them with water.

Appendix B

Causes Analysis of Abnormalities

If it is necessary to find out the reasons for swell, pH or organoleptic abnormalities, and microorganism proliferation, get the reserved sample of the canned food for inoculation and cultivation, and then report.

In order to judge whether the can has leakage, get the opened can for tightness check and then report.

B.1 Terms and Definitions

B.1.1 Low acid canned food

Besides alcohol, all canned food whose balance pH is higher than 4.6 and water activity larger than 0.85 after sterilization is low acid canned food. When low acid fruits, vegetables or vegetable products are canned, acid is added to decrease the pH in order to meet the requirement of heat sterilization, and these canned foods belong to acidified low acid canned food.

B.1.2 Acid canned food

Acid canned food refers to canned food whose balance pH equals or is less than 4.6 after sterilization. Tomatoes, pears, pineapples and their juices with pH value less than 4.7, and figs whose pH is less than 4.9 are all considered as acid food.

B.2 Equipments and materials

Besides the regular sterilization and culture equipments used in microbiological labs, Other equipments and materials are as follows:

B.2.1 Constant temperature incubator: 30℃ ± 1℃; 55 ℃ ± 1℃.

B.2.2 Constant temperature water bath: 55℃ ± 1℃.

B.3 Culture medium and reagent

B.3.1 Bromocresol purple dextrose broth

B.3.1.1 Ingredients

Peptone	10.0 g
beef extract	3.0 g
dextrose	10.0 g
sodium chloride	5.0 g
bromcresol purple	0.04 (or 1.6% alcoholic solution 2.0mL)
distilled water	1,000.0mL

B.3.1.2 Preparation

Heat and stir the above-mentioned ingredients (except bromocresol purple) to make them dissolve, adjust the pH value to 7.0 ± 0.2, and add in bromocresol purple. Put the solution into several medium-sized test tubes with small discharging tubes, 10 mL for each test tube. Then these test tubes shall go through high-pressure sterilization for 10 minutes under the temperature of 121℃.

B.3.2 Cooked meat medium

B.3.2.1 Ingredients

beef infusion	1,000.0 mL
peptone	30.0 g
yeast extract	5.0 g
dextrose	3.0 g
sodium dihydrogen phosphate	5.0 g
soluble starch	2.0 g
meat fragments	appropriate amount

B.3.2.2 Preparation

B.3.2.2.1 Get 500g fresh beef fragments free of fat and fascia, add in 1,000mL distilled water and 25.0 mL 1moL/L sodium hydroxide solution, boil the solution for 15 minutes, and then cool it completely. Get rid of the fat on the surface, settle and filter the solution, and add in more water to 1000 mL. Add in all the ingredients except meat fragments, and adjust the pH value to 7.8 ± 0.2.

B.3.2.2.2 Wash the meat fragments with water, air them to semi-dry state, and put them into several 15 mm × 150 mm test tubes, meat fragments in each tube reaching 2 cm ~ 3 cm high. Add 0.1 g ~ 0.2 g reduced iron powder or a small amount of scrap iron to each test tube. Pour the fluid nutrient medium mentioned above into each test tube, and the fluid nutrient medium shall be 1 cm higher above the surface of the meat fragments. Finally, add dissolved vaseline or liquid paraffin 0.3 cm ~ 0.4 cm to each test tube. Then these test tubes shall go through sterilization for 15 minutes under the temperature of 121℃

B.3.3 **Nutrient agar**

B.3.3.1 **Ingredients**

peptone	10.0 g
beef extract	3.0 g
sodium chloride	5.0 g
agar	15.0 g ~ 20.0 g
distilled water	1,000.0 mL

B.3.3.2 **Preparation**

Let all the above ingredients except agar dissolve in the distilled water, add in 2 mL 15% sodium hydroxide solution, and adjust the pH value to 7.2 ~ 7.4. Add in the agar, heat the solution until it boils and the agar dissolves. Put the solution into several flasks or 13 mm × 130 mm test tubes. The flasks or test tubes shall then go through high-pressure sterilization for 15 minutes under the temperature of 121 ℃.

B.3.4 **Acid broth**

B.3.4.1 **Ingredients**

polypeptone	5.0 g
yeast extract	5.0 g
dextrose	5.0 g
monopotassium phosphate	5.0 g
distilled water	1,000.0 mL

B.3.4.2 **Preparation**

Heat and stir the above ingredients to make them dissolve, adjust the pH value to 5.0 ± 0.2, and then sterilize it with high pressure for 15 minutes under the temperature of 121℃.

B.3.5 **Malt extract broth**

B.3.5.1 **Ingredients**

malt extract	15.0 g
distilled water	1,000.0mL

B.3.5.2 **Preparation**

Let the malt extract completely dissolve in the distilled water, filter the solution with filter paper, adjust the pH value to 4.7 ± 0.2, and put the solution into several containers, which shall then be sterilized for 15 minutes under the temperature of 121℃.

B.3.6 Sabouraud's dextrose agar

B.3.6.1 **Ingredients**

peptone	10.0 g
agar	15.0 g
dextrose	40.0 g
distilled water	1,000.0 mL

B.3.6.2 **Preparation**

Let all the ingredients dissolve in the distilled water, heat the solution, put the solution into several flasks, and adjust the pH value to 5.6 ± 0.2. These flasks shall go through high-pressure sterilization for 15 minutes under the temperature of 121 ℃.

B.3.7 Calf liver agar

B.3.7.1 **Ingredients**

calf liver (used to make calf liver infusion)	50.0 g
veal (used to make veal infusion)	500.0 g
proteose peptone	20.0 g
new peptone	1.3 g
tryptone	1.3 g
dextrose	5.0 g
soluble starch	10.0 g
plasma casein protein	2.0 g
sodium chloride	5.0 g
sodium nitrate	2.0 g
gelatin	20.0 g
agar	15.0 g
distilled water	1,000.0 mL

B.3.7.2 **Preparation**

Mix all the above-mentioned ingredients in the distilled water and sterilize the solution in 121℃ for 15 minutes. The final pH value shall be 7.3 ± 0.2.

B.3.8 Gram staining solution

B.3.8.1 Crystal violet staining solution

B.3.8.1.1 Ingredients

Crystal violet	1.0 g
95% ethanol	20.0 mL
1% ammonium oxalate aqueous solution	80.0 mL

B.3.8.1.2 Preparation

Let the crystal violet totally dissolve in ethanol, and then mix the ethanol with the ammonium oxalate solution.

B.3.8.2 **Gram iodine solution**

B.3.8.2.1 **Ingredients**

iodine	1.0 g
potassium iodide	2.0 g
distilled water	300 mL

B.3.8.2.2 **Preparation**

Mix iodide and potassium iodide firstly, add a small amount of distilled water and shake it adequately; after full dissolution, add distilled water to 300 mL.

B.3.8.3 **Safranine re-staining solution**

B.3.8.3.1 **Ingredients**

safranine	0.25 g
95% ethanol	10.0 mL
distilled water	90.0 mL

B.3.8.3.2 **Preparation**

Dissolve safranine into the ethanol and then dilute it with distilled water.

B.3.8.4 **Staining method**

a) Smear is fixed above the fire, add crystal violet staining solution in droplets to stain it for 1 minute, wash the smear with water.

b) Add Gram iodine solution in droplets, and wash the smear with water after 1 minute.

c) Add 95% ethanol in droplets to make decoloration for about 15 seconds~30 seconds until the staining solution is scrubbed down, no excessive decoloration, wash the smear with water.

d) Add re-staining solution in droplets for 1 min, wash the smear with water, and examine it with microscope after drying.

B.4 Inoculation and cultivation of low acid food (pH value >4.6)

B.4.1 For low acid food, inoculate four tubes each can into cooked meat medium that has been heated to 100℃and then cooled to the room temperature rapidly, meanwhile, inoculate four tubes of bromocresol purple dextrose broth.

Inoculate 1 mL (g)~2 mL (g) sample (liquor sample is 1 mL~2 mL, solid is 1 g~2 g, when both exist, take half of each) for each tube. See Table B.1 for culture conditions.

Table B.1 Low acid food (pH value > 4.6) Inoculated cooked meat medium and bromocresol purple dextrose broth

Culture medium	Tube Quantity.	Culture temperature(℃)	Culture time(h)

Cooked meat medium	2	36 ± 1	96 ~ 120
Cooked meat medium	2	55 ± 1	24 ~ 72
Bromocresol purple dextrose broth	2	55 ± 1	24 ~ 48
Bromocresol purple dextrose broth	2	36 ± 1	96 ~ 120

B.4.2 After being cultured in the culture conditions stipulated in Table B.1, make record whether there are microorganisms grown in each tube. If not, abandon it after recording.

B.4.3 If there are microorganisms growing on it, stain solution smear with inoculating loop, examine the Gram staining with microscope. If different microorganism forms or unitary cocci and fungus forms are observed in the tube of bromocresol purple dextrose broth, record them before abandoning them. If no bacillus found in cooked meat medium and coccus, yeast, mould or its mixture are found in the culture, record them and then abandon. Make streak inoculation of two calf liver agar (without yolk) or other nutrient agar plate respectively in positive tubes appearing and growing in bromocresol purple dextrose broth and cooked meat medium; one plate for aerobic culture and the other for anaerobic culture. (As shown in Table B.1)

B.4.4 Select single colony in aerobic culture and inoculate it on the small slant of nutrient agar for subsequent examine the Gram staining with microscope. Select single colony smear in anaerobic culture, and examine the Gram staining with microscope. Select the single colony in aerobic and anaerobic culture and inoculate it in cooked meat medium to do purely culturing.

B.4.5 Select the culture smear in nutrient agar small slant and cooked meat medium of anaerobic culture for microscopic examination.

B.4.6 Select the aerobic culture in purely culturing and inoculate it with calf liver agar (without yolk) or nutrient agar plate to carry out anaerobic culture; select anaerobic culture in purely culturing and inoculate it with calf agar (without yolk) or nutrient agar plate to carry out aerobic culture, so as to distinguish whether they are facultative anaerobes.

B.4.7 If it is necessary to detect botulin of clostridia, select typical colony to inoculate cooked meat medium to make pure culture. Culture under the temperature of 36 ℃ for 5days, and botulin examination is carried out according to GB/T 4789.12.

B.5 Inoculation and culture of acid food(pH value ≦ 4.6)

B.5.1 Each can is inoculated with four tubes of acid broth and two tubes of malt extract broth. Inoculate 1 mL (g)~2 mL (g) sample (the volume of liquor sample 1 mL~2 mL and solid 1 g~2 g, when both of them exist, take half of each) for each tube. See Table B.2 for culture conditions.

Table B.2 Acid broth and malt extract soup inoculated in acid food (pH value ≤ 4.6)

Culture medium	Tube Quantity.	Culture temperature (℃)	Culture time (h)
Acid broth	2	55 ± 1	48
Acid broth	2	30 ± 1	96

Malt extract broth	2	30 ± 1	96

B.5.2 After being cultured in the culture conditions stipulated in Table B.2, make record whether there are microorganisms grown in each tube. If not, record them and then abandon.

B.5.3 For culture tube with microorganism grown in, take the direct smear of the culture after culturing, make the Gram staining microscopic examination and record the observed microorganisms.

B.5.4 If there are microorganisms grown in acid broth or malt extract broth in 30℃ culture conditions, inoculate each positive tube with two pieces of nutrient agar or Sabouraud's dextrose agar plate respectively, one plate for aerobic culture and the other for anaerobic culture.

B.5.5 If there are microorganisms grown in acid broth in the culture conditions of 55℃, inoculate each positive tube with two pieces of nutrient agar plates respectively, one for aerobic culture and the other for anaerobic culture (as shown in Table B.2). Make staining smear microscopic examination of the plate where microorganisms grown and report the type of the microorganism found in the microscopic examination.

B.5.6 Select single colony of nutrient agar or Sabouraud's dextrose agar plate cultured in 30 ℃ aerobic culture condition, inoculate nutrient agar small slant for the subsequent Gram staining microscopic examination. Meanwhile, inoculate acid broth or malt extract broth to make pure culture.

Select single colony of nutrient agar or Sabouraud's dextrose agar plate cultured in 30℃ anaerobic culture condition, inoculate acid broth or malt extract broth to make pure culture.

Select single colony of nutrient agar plate in 55℃ aerobic culture condition, inoculate nutrient agar small slant for the subsequent Gram staining microscopic examination. Meanwhile, inoculate acid broth to make pure culture.

Select single colony of nutrient agar plate in 55℃ anaerobic culture condition, inoculate acid broth to make pure culture.

B.5.7 Select culture in nutrient agar small slant to make smear microscopic examination; select acid broth or malt extract broth culture in 30℃ anaerobic culture condition and acid broth culture in 55℃ anaerobic culture condition to make smear microscopic examination.

B.5.8 Inoculate the pure culture which is cultured in 30℃ aerobic culture condition in nutrient agar or Sabouraud's dextrose agar plate to make anaerobic culture; inoculate the pure culture cultured in 30℃ anaerobic culture condition in nutrient agar or Sabouraud's dextrose agar

plate to make aerobic culture; inoculate the pure culture cultured in 55 ℃ aerobic culture condition in nutrient agar to make anaerobic culture; inoculate the pure culture cultured in 55℃ anaerobic culture condition in nutrient agar to make aerobic culture to distinguish whether they are facultative anaerobes.

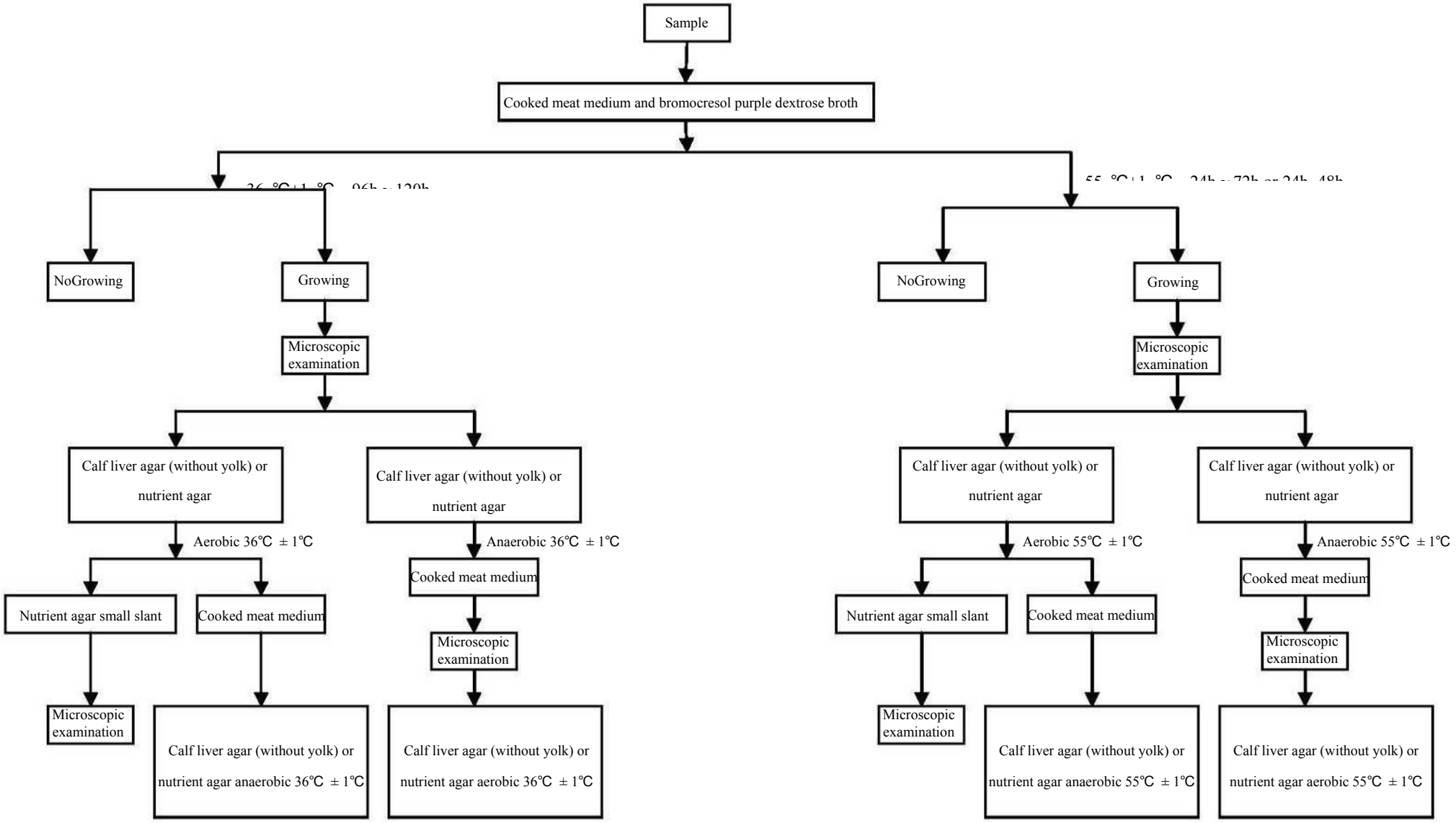

Figure B.1 Low-acid Food Vaccination Cultivation Procedure

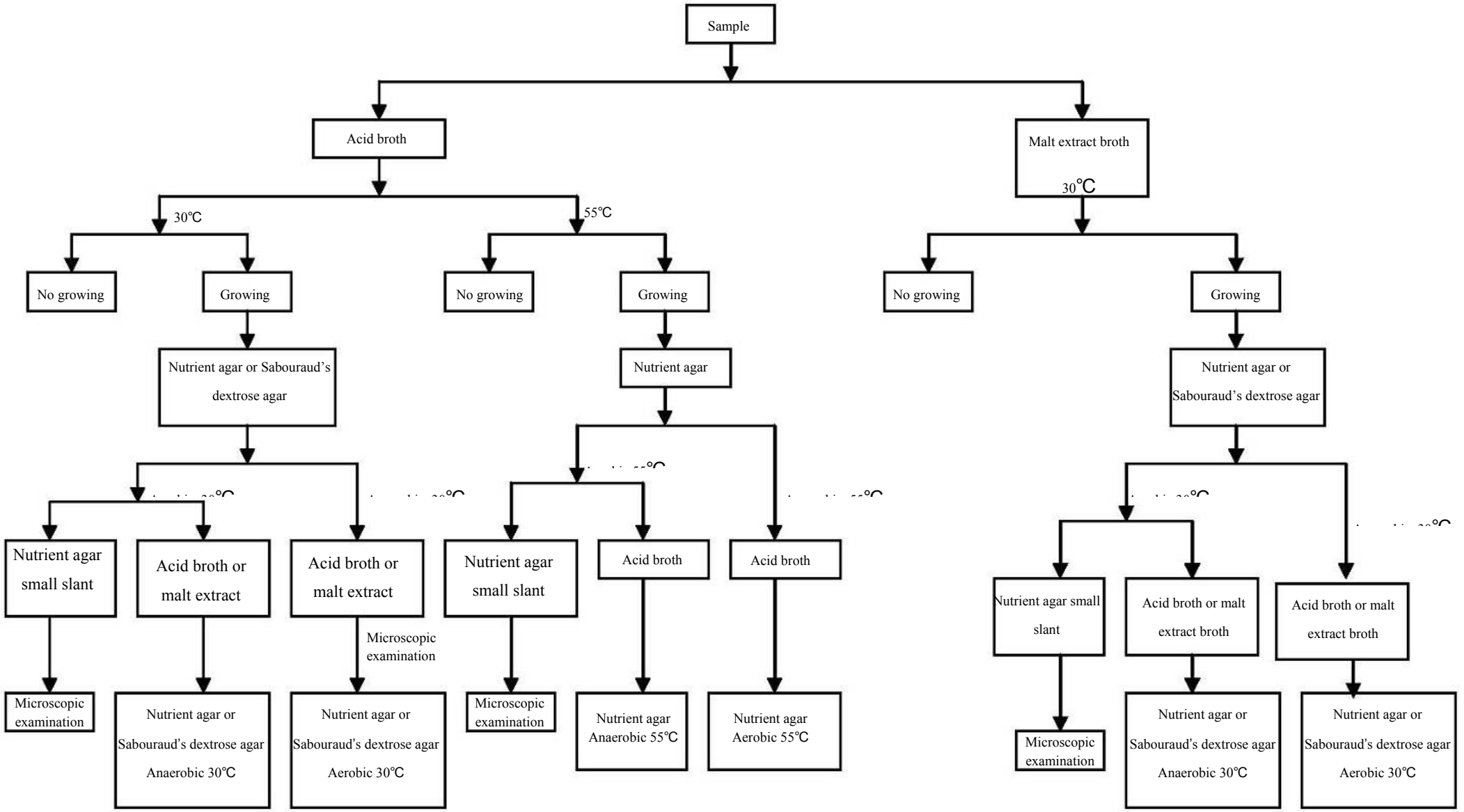

Figure B.2 Acid Food Inoculation Cultivation Procedure

B.5.9 Results Analysis

B.5.9.1 If no growth of microorganism was found in the swelled can, it may because the contents and the package react and produce hydrogen. The amount of hydrogen produced changes with the storage time and conditions. Excessive filling of the can also causes slight swell, which can be confirmed through weighting.

If mixed microorganisms of large amount of bacteria is found in direct smear which does not grow after cultivation, it indicates the putrefaction occurred before canning. As the result of bacterial growth before canning, pH, odor and morphology of the product are in abnormal conditions.

B.5.9.2 When the can is well sealed and only bacillus grow under the culture conditions of 36℃ whose heat resistance is not higher than botulinum clostridia, it indicates that the heat is not sufficient in the production process.

B.5.9.3 If mixed colonies of bacilli, cocci and fungi are cultivated, it shows that the can leaks. It may also be due to the incomplete sterilization. Under this condition the swell rates of the same batch of product shall be higher.

B.5.9.4 If acid is produced under the condition of bromocresol purple dextrose broth at 36℃ or 55℃ to be cultivated to observe gas and acid production, it indicates that there is growth of microorganism addicted to proper temperature, for example, mesophilic acid-resistant bacillus, or thermophilic microorganisms such as bacillus stearothermophilus.

If the bacteria grow , produce gas and give rotten smell under the cooked meat medium under the temperature of 55℃, it indicates that the putrefaction in the can is caused by thermophilic anaerobic clostridia.

If the gas grows out of the cooked meat medium under the temperature of 36℃ with rotten smell and the germ can be seen from microscopic examination, this indicates that the putrefaction is caused by botulinum clostridia, sporogenic fusiform bacillus or clostridium perfringens. Further botulinum detection can be carried out when necessary.

B.5.9.5 Deterioration of acid food is usually caused by lactobacillus and yeast without spore.

Generally, the deterioration caused by bacillus shall not happen when pH is lower than 4.6. But there is an exception, i.e. the deteriorated catsup or tomato juice can displayed as sour spoilage with rotten smell and with or without the reduce of pH are generally caused by aerobic bacillus.

B.5.9.6 Many canned food contains thermophile bacteria which does not grow in normal storage condition but grows and causes putrefaction when exposed to higher temperature (50℃～55℃). Thermophilic acid-resistant bacillus and bacillus stearothermophilus will cause sour spoilage putrefaction in acid and low acid food respectively. Culture at the temperature of 55 ℃ shall not cause the appearance change of the canned food but shall produce rotten smell with or without the reduce of pH. Sometimes the putrefaction of canned food of tomatoes, pears, figs, pineapples and other canned food is caused by clostridium pasteurianum (a kind of anaerobic bacterium that can produce acid and gas). Thermophilic sucroclastic clostridium is a kind of thermophilic anaerobic

bacteria which can cause swell and rotten smell of the product.

Thermophilic anaerobic bacteria can also produce gas. For the bacteria rapidly proliferates after beginning to grow, the confounded swell may be caused by hydrogen or the production of gas of thermophilic anaerobic bacteria. The decomposition of chemical shall produce carbon dioxide, which especially happens among the food containing sugar and some acid food such as canned food of catsup, molasses, mincemeat and fruit with high sugar. The decomposition speeds up with the rise of the temperature.

B.5.9.7 Any microorganism isolated from vacuum packing of sterilization and normal product direct smear shall be suspected to be due to laboratory pollution. In order to prove whether it is laboratory pollution, the isolated and living microorganism shall be inoculated to another normal control can, hermetical sealed and cultured at the temperature of 36℃ for 14 days. If swell or product putrefaction occurs, these microorganisms may not come from original sample. If the can is still flat, open the can by aseptic technique and culture as the above-mentioned procedure. If the same kind of microorganism is found again and the product is normal, the product is deemed as commercial sterilization for this kind of microorganism does not grow in the normal storage and shipment.

B.5.9.8 If the food itself is turbid, the definitive conclusion may not be obtained by broth cultivation. Under this condition further cultivation shall be carried out to confirm whether there grow microorganisms.

B.6 Detection Method of Can Tightness

The cleaned empty can after drying under the temperature of 36℃ can be selected to carry out the decompression and pressurization leakage check.

B.6.1 Decompression leakage check

Carefully inject clean water with the fullness of 80%- 90% into the empty can and properly place an organic glass with rubber band at the roll rim on open end of the can which can keep the can tight. Start up vacuum pump, close air bleeder, press the cover plate with hands, control air exhaust to make the time of vacuum meter rising from 0 Pa to 6.8×10^4Pa（510 mmHg）more than 1 minute and keep this vacuum degree for more than 1 minute. Tilt and carefully observe whether can body, especially hemming and welding line produce bubbles. If the bubbles are produced continuously from the same position, it shall be judged as leakage. Record the leakage time and vacuum degree and mark the leakage position.

B.6.2 Pressurization Leakage Check

Stuff up the open end of the empty can by rubber stopper, immerse the empty can in the glass jar which is filled with water, start up air compressor, open the valve slowly to make the pressure in the can increase gradually until the pressure rising to 6.867×10^4 Pa and keep it for 2 minutes. Carefully observe whether the can body, especially hemming and welding line produces bubbles. If the bubbles are produced continuously from the same position, it shall be judged as leakage. Record the leakage time and vacuum degree and mark the leakage position.