National Standard of the People's Republic of China

GB 4789.26-2013

National Food Safety Standard

Food Microbiological Examination Commercial Sterilization Examination

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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Foreword

The standard replaces GB/T 4789.26---2003 Food Hygiene Microbiological Examination Commercial Sterilization Examination of Canned Food.

Compared with GB/T 4789.26--2003, the main changes are as follows:

— The Chinese name of the Standard are revised;

— The scope is revised;

— Normative references are deleted;

— Terms and definitions are revised;

— Equipment and materials are revised;

— The culture medium and reagents are revised;

— Inspection procedure charts are added;

— Test steps are revised;

— Result determination is revised;

— Appendix A and Appendix B are revised.

National Food Safety Standard

Food Microbiological Examination Commercial Sterilization Examination

1. Scope

This standard stipulates the basic requirements, operation procedures and result determination of the commercial sterilization examination of food.
This standard is applicable to the commercial sterilization examination of food.

2. Terms and definitions

The following terms and their definitions are applicable to the Standard.
2.1 Low acid canned foods
In addition to alcoholic beverages, the acidulated low-acid canned foods are made from low-acid fruit, vegetables or vegetable products, pH value of which are reduced by adding acid for the purpose of heat sterilization, whose equilibrium pH value should be greater than 4.6 after being sterilized and the water activity should be more than 0.85 belong to low acid canned foods.
2.2 Acid canned foods
The canned foods of which the equilibrium pH values are equal to or below 4.6 after being sterilized belong to acid canned foods. Tomatoes, pears, pineapples and juices processed from them of which pH values are below 4.7 as well as figs with the pH value of below 4.9 are acid canned foods.

3. Equipment and materials

Besides the regular equipment for sterilization and culture in microbiology lab, other equipment and materials required are as follows:

a) Refrigerator: 2℃~5℃;

b) Constant temperature incubator: 30℃±1℃; 36℃±1℃; 55℃±1℃;

c) Constant temperature water bath case: 55℃±1℃;

d) Homogenizer, homogeneous bag, homogeneous cup and pestle;
e) Potential pH meter (accuracy:pH0.05 unit);
f) Microscope (10~100-fold);
g) Can opener and puncher for can;
h) Electronic scale or bench balance;
i) Super clean bench or Class 100 clean lab.

4. Culture media and reagents

4.1 Sterile saline solution: see A.1 in appendix A.
4.2 Crystal violet staining solution: see A.2
4.3 Dimethylbenzene
4.4 Ethanol solution containing 4% iodine: dissolve 4 g iodine in 100mL of 70% ethanol solution.

5. Inspection procedures

Commercial sterilization inspection procedures are shown in Figure 1.

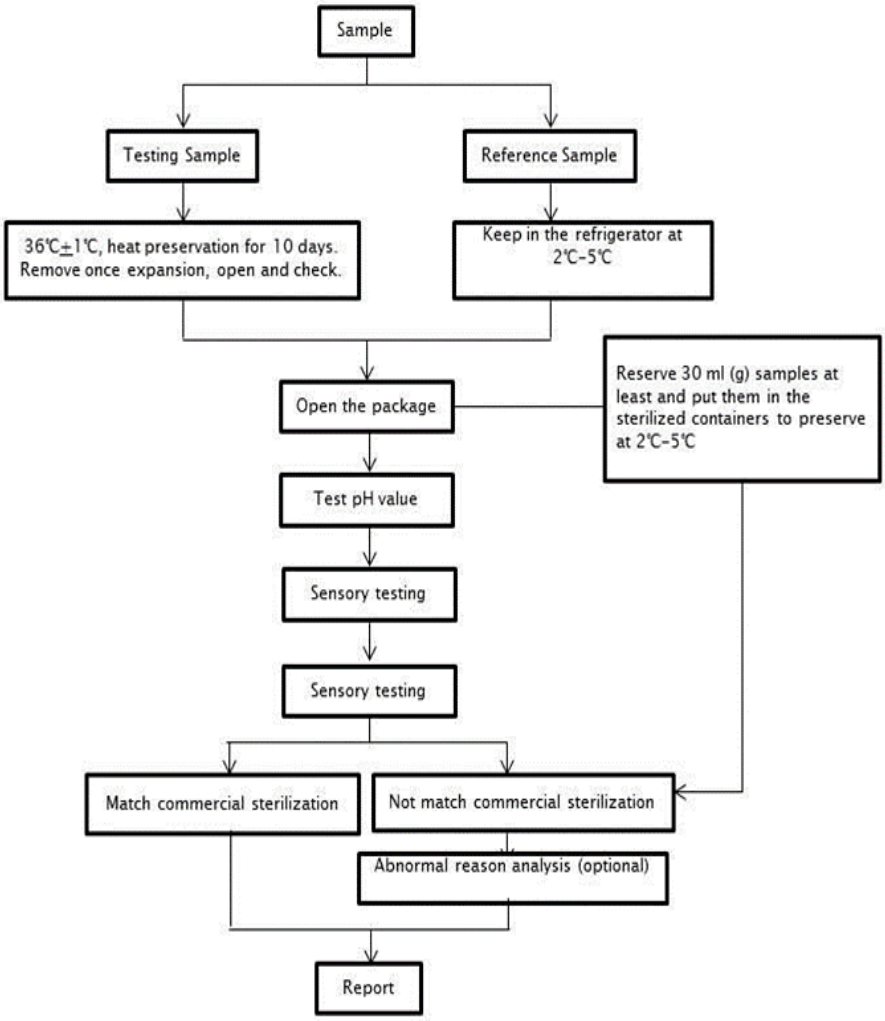

Figure 1 Inspection procedures of commercial sterilization

6. Operation steps

6.1 Samples preparation

Remove the external tags, and use a waterproof permanent marker to mark on the surface of the container. Record the container, serial number, product characters and leakage as well as abnormal conditions, such as holes on the container or rust, indentation and expansion and so on.

6.2 Weight

Packed objects of 1 kg or less should be weighed with an accuracy to 1 g, those of more than 1 kg should be weighed with an accuracy to 2 g and those over 10kg should be weighed with an accuracy to 10g. At the same time, make records of them.

6.3 Heat Preservation

6.3.1 Take one sample from each batch, store them in the refrigerator at 2℃-5℃ as references, and preserve the other samples for 10 days under the temperature of 36℃±1℃. During heat preservation, daily checks should be conducted and the dilatant or leaked ones should be taken out immediately for inspection once detected.

6.3.2 Weigh again and make records when heat preservation is finished, and compare the weights before and

after the heat preservation to check for any changes. The lighter weights of samples indicate that leakage occurs in samples. On such conditions, keep all packed objects under room temperature until they are unpacked for inspection.

6.4 Unpack

6.4.1 Keep the dilatant samples in the refrigerator at 2℃-5℃ for several hours before opening.

6.4.2 If expansions occur, clean the smooth surface of samples to be tested with cold water and detergent. After being washed by water, dry the smooth surface with sterile towels. Soak the smooth surface in the ethanol solution containing 4% iodine for 15 minutes to disinfect, then dry it with sterile towels, and light it in airtight cover until the ethanol solution containing iodine remained on the surface is burned off. Don't burn the dilatant samples and those packed with combustible packaging materials, and just soak the smooth surface in the ethanol solution containing 4% iodine for 30 minutes, and dry it with sterile towels.

6.4.3 Unpack the samples on the clean bench or in the Class 100 clean lab. Before that, the samples with soup should be properly shaken. Open a hole of appropriate size on the smooth surface of the sterilized can with a sterile opener, and breaking the crimping structure is not allowed during this process. One opener shall be used by only one can, and cross-use shall be banned. If samples are packed with soft materials, they could be open with sterile scissors and the seams should not be broken. Sniff the smell immediately after opening and make a record.

Note: The seriously dilatant samples may explode and toxic substances may spray. Such hazardous events could be prevented by covering the dilatant samples with sterile towels or sterile funnel.

6.5 Sample reserving

After the unpacking, take out at least 30 ml (g) of the contents with sterile straws or other proper tools and put in sterile containers through sterile operations. Store the contents in the refrigerator at 2℃-5℃ for further test if necessary. After testing conclusion of the batch of samples is achieved, the contents could be thrown away. The unpacked samples should be properly preserved for future examinations on the containers.

6.6 Sensory test

In the test room with sufficient light, clean air and no bad smell, pour the contents of samples into white enamel dish, then observe the structure, shape and color, sniff the odor, and press the food to inspect its properties, and so that judgments could be made if the food has been spoiled. At the same time, observe the packaging containers from outside and inside and make records at the same time.

6.7 Determination of pH values

6.7.1 Sample treatment

6.7.1.1 Mix the liquid products for further use. As for the products with both solid and liquid phase, choose the well mixed liquid-phase part for use.

6.7.1.2 For the thick or half-thick products or those from which it's difficult to extract juice (such as syrup, jam, jelly, grease and so on), take part of them and grind in a homogenizer or mortar. If the samples are still too thick after being grinded, add same amount of sterile distilled water into them and fully mix for use.

6.7.2 Determination

6.7.2.1 As the solutions to be tested, insert the electrodes into the sample solutions to be tested, and adjust the temperature corrector of pH meter to the same temperature. Adjust the temperature of solutions to be tested to 20℃±2℃ and conduct determination by the steps applicable for all pH meters if temperature correction system is not available,. After the numerical readings become steady, directly read the pH values from the scales, with the accuracy to 0.05pH unit.

6.7.2.2 The same prepared sample should be determined at least twice, and the differences between two results should not exceed 0.1pH unit. Take the arithmetic mean value of the two tests as the result, and report result shall be with the accuracy to 0.05pH unit.

6.7.3 Result analysis

Compare the results with the refrigerated control samples of the same batch to check for obvious differences between them. The differences between pH values reaching 0.5 or above can be determined as obvious ones.

6.8 Smear staining microscopy

6.8.1 Smear

Take the contents of samples to make smears. For samples with soup, take out some soup from which by an inoculating loop, and smear it on glass slides. Solid products can be used to make into smears directly or be diluted by small amount of stroke-physiological saline solution (SPSS) and then made into smears. Then it should be fastened with fire after drying. For greasy food its smears should be dried naturally and fastened with fire, then washed with flowing dimethylbenzene and dried naturally.

6.8.2 Staining microscopic examination

The smears in 6.8.1 should be conducted simple staining with crystal violet staining solution and carry out microscopic examination for them after drying. Observe at least five fields of microscope and record mycelial morphology and the bacterial count within each field. Compare them with refrigerated samples of the same batch to see if obvious microbial proliferation occurs. Obvious proliferation is indicated if the bacterial count grows a hundred fold or above.

7. Interpretation of Results

The product can be reported as commercial sterilization if there is no leakage within the samples after heat preservation test, and no microbial proliferation is observed in sensory test, pH value determination and smear microscopic examination.

The product can be reported as non-commercial sterilization, if there are leakages within samples after heat preservation test, and microbial proliferations are observed in sensory test, pH value determination and smear microscopic examination.

If it's necessary to find out the reasons for expansion, unusual pH values or sensory experience and microbial proliferation, take the reserved samples to inoculate and culture and make a report as per Appendix B. When it is necessary to determine whether there is a leakage occurs in the containers of samples, conduct a sealing test for the unpacked samples and make a report of it.

Appendix A

Culture Medium and Reagent

A.1 Stroke-physiological saline solution

A.1.1 Compositions

Sodium chloride	8.5g
Distilled water	1000.0mL

A.1.2 Preparation method

Weigh 8.5g of sodium chloride and dissolve in 1000ml of distilled water. Autoclave at 121℃ for 15 minutes.

A.2 Crystal violet staining solution

A.2.1 Compositions

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

A.2.2 Preparation method

Dissolve 1.0g of Crystal Violet in 95% ethanol, and then mix with 1% ammonium oxalate solution.

A.2.3 Staining method

Fix the smear with the flame from an alcohol burner. Add crystal violet to the smear drop by drop and stain for 1 min, then rinse with water.

Appendix B

Abnormal Cause Analysis (Optional Item)

B.1 Culture medium and reagent

B.1.1 Bromcresol purple dextrose broth

B.1.1.1 Compositions

Peptone	10.0g
Beef extract	3.0g
Glucose	10.0g
Sodium chloride	5.0g
Bromocresol Purple	0.04g (or 1.6% ethanol solution, 2.0mL)
Distilled water	1000.0mL

B.1.1.2 Preparation method

Heat, mix and dissolve all the compositions excluding Bromocresol Purple. Adjust pH to 7.0±0.2 and then add Bromocresol Purple into the mixed solution. Dispense it into test tubes with small inversed test tubes. And each tube should be filed withl 10mL of the mixed solution and autoclaved at 121℃ for 10 minutes.

B.1.2 Cooked meat medium

B.1.2.1 Compositions

Beef infusion broth	1000.0mL
Peptone	30.0g
Yeast extract	5.0g
Glucose	3.0g
Sodium dihydrogen phosphate	5.0g
Soluble starch	2.0g
Meat residue	appropriate amount

B.1.2.2 Preparation method

B.1.2.2.1 Weigh 500g of fresh minced beef without fat and fascia, then mixed with 1000mL of distilled water and 25.0mL of 1moL/L sodium hydroxide solution. Stir the solution and boil it for 15 minutes, and cool it down. Remove the external fat, clarify and filter the solution. Add 1000mL of water to get the beef infusion broth. Add in all the compositions except meat residue referred to in B.1.2.1 , and then adjust the pH value to 7.8±0.2.

B.1.2.2.2 Wash the meat residue with water and make it half-dry. Respectively fill it into test tubes (15mm×150mm) which is about 2cm to 3cm high. Add 0.1g to 0.2g reduced iron powder or a few iron filings into each test tube. Fill every test tube with the prepared liquid culture medium of B.1.2.2.1, making the liquid about 1cm higher than the surface of meat residue. It is then topped with dissolved petrolatum or liquid paraffin (0.3cm to 0.4cm high). Autoclave at 121℃ for 15 minutes.

B.1.3 Nutrient agar

B.1.3.1 Compositions

Peptone	10.0g
Beef extract	3.0g
Sodium chloride	5.0g
Agar	15.0g - 20.0g
Distilled water	1000.0mL

B.1.3.2 Preparation method

Dissolve all the compositions except agar in distilled water, and then add in about 2mL of 15% sodium hydroxide solution. Adjust pH to 7.2 to 7.4. Then add in agar and heat to boiling to dissolve agar. Dispense the obtained solution into flasks or test tubes (13mm×130mm) respectively. Autoclave at 121℃ for 15 minutes.

B.1.4 Acid broth

B.1.4.1 Compositions

Polypetone	5.0g
Yeast extract	5.0g
Glucose	5.0g
Potassium dihydrogen phosphate	5.0g
Distilled water	1000.0mL

B.1.4.2 Preparation method

Heat, stir and dissolve all the compositions referred to in B.1.4.1. Adjust pH to 5.0±0.2. Autoclave at 121℃ for 15 minutes.

B.1.5 Malt extract broth

B.1.5.1 Compositions

Malt extract	15.0g
Distilled water	1000.0mL

B.1.5.2 Preparation method

Dissolve malt extract fully in distilled water, then filter by filter paper. Adjust pH to 4.7±0.2 and dispense it to several containers. Autoclave at 121℃ for 15 minutes.

B.1.6 Sabouraud's dextrose agar

B.1.6.1 Compositions

Peptone	10.0g
Agar	15.0g
Glucose	40.0g
Distilled water	1000.0mL

B.1.6.2 Preparation method

Dissolve all the compositions in distilled water and heat to boiling. Adjust pH to 5.6±0.2. Autoclave at 121℃ for 15 minutes.

B.1.7 Liver veal agar

B.1.7.1 Compositions

Liver extract	50.0g
Veal extract	500.0g
Proteose peptone	20.0g
Neopeptone	1.3g
Tryptone	1.3g
Glucose	5.0g
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

B.1.7.2 Preparation method

Dissolve all the compositions in distilled water. Adjust pH to 7.3±0.2. Autoclave at 121℃ for 15 minutes.

B.1.8 Gram stain solution

B.1.8.1 Crystal violet staining solution

B.1.8.1.1 Compositions

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

B.1.8.1.2 Preparation method

Dissolve 1.0g of crystal violet fully in 95% ethanol and then mix with 1% ammonium oxalate solution.

B.1.8.2 Gram's iodine solution

B.1.8.2.1 Compositions

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300mL

B.1.8.2.2 Preparation method

First, mix 1.0g of iodide with 2.0g of potassium iodide. Then add a little distilled water into the mixture and shake

well. After it is fully dissolved, add distilled water to 300mL.

B.1.8.3 Safranine (counterstain)

B.1.8.3.1 Compositions

Safranine	0.25g
95% Ethanol	10.0mL
Distilled water	90.0mL

B.1.8.3.2 Preparation Method

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

B.1.8.4 Staining Method

B.1.8.4.1 Fix a smear on the flame from an alcohol burner. Add crystal violet to the smear drop by drop and stain for 1 min, then rinse with water.

B.1.8.4.2 Add gram's iodine drop by drop, retain for 1 minute and then rinse with water.

B.1.8.4.3 Add 95% ethanol drop by drop, decolor for about 15~30s until the staining solution is washed off. Decoloring too much is not allowed, and then rinse with water.

B.1.8.4.4 Add counterstain liquid drop by drop, re-dye for 1 minute and rinse with water. Conduct microscopy after drying.

B.2 Inoculated Culture of Low-acid Canned Food (pH>4.6)

B.2.1 For low-acid canned food, inoculate each of its sample in 4 test tubes of cooked meat medium preheated to 100℃and rapidly cooled down to room temperature; Meanwhile, inoculate in 4 test tubes of bromcresol purple dextrose broth. Inoculate 1mL(g)~2mL(g) of samples (1 mL~2 mL for liquid samples, 1g~2g for solid samples, if both exist, half of each for both) in each test tube. The conditions of culture are shown in Table B.1.

Table B.1 Cooked Meat Medium and Bromcresol Purple Dextrose Broth for Inoculation of Low-acid Canned Food (pH>4.6)

Culture Medium	Number of Tubes	Culture Temperature/ ℃	Culture Time/ h
Cooked Meat Medium	2	36±1	96~120
Cooked Meat Medium	2	55±1	24~72
Bromcresol Purple Dextrose Broth	2	55±1	24~48
Bromcresol Purple Dextrose Broth	2	36±1	96~120

B.2.2 After culturing in accordance with the conditions shown in Table B.1, record states of microbial growth in each tube. If no microbial growth is observed in a tube, discard it after recording.

B.2.3 If there is microbial growth in a tube, pick liquid with an inoculating loop to make a smear and conduct Gram staining microscopy for it. If different microbial morphologies or a single coccal or fungal morphology is observed in a tube of bromcresol purple dextrose broth, then discard it after recording. If there is no bacillus, and yeast, mycete and their mixtures are observed in a tube of cooked meat medium, discard it after recording. Inoculate the other positive tubes of bromcresol purple dextrose broth and cooked meat mediumin which microbial growth is observed on two liver veal agar or nutrient agar streak plates, one for aerobic cultivation, another for anaerobic cultivation. The cultivation shall refer to Figure B.1.

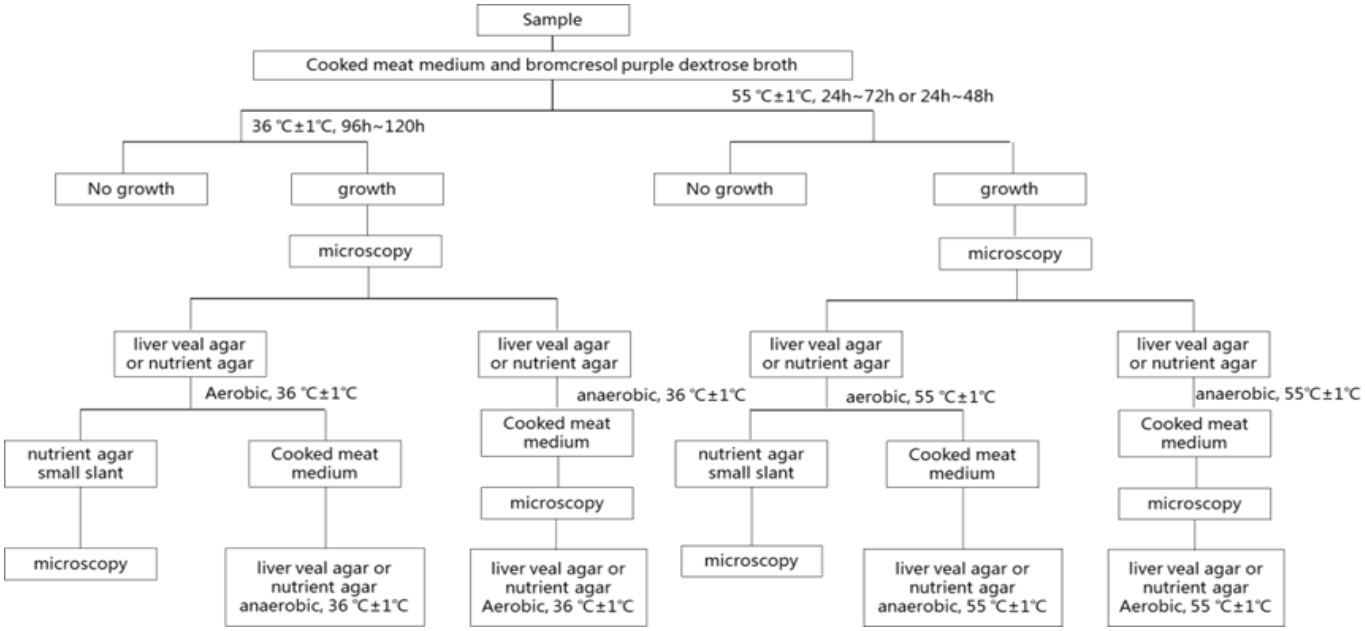

Figure B.1 The Procedures of the Inoculation and Culture of Low-acid Canned Food

B.2.4 Pick a single colony in aerobic culture and inoculate it on the nutrient agar small slant, which will be used for the later Gram's staining microscopic examination. Pick a single colony in anaerobic culture to make a smear and conduct Gram's staining microscopy for it. Inoculate single colonies respectively picked from aerobic and anaerobic culture into cooked meat medium to conduct pure culture.

B.2.5 Pick cultures from nutrient agar small slant and anaerobically cultured cooked meat medium to make a smear and conduct microscopy for it.

B.2.6 Pick aerobic cultures from pure cultivation and inoculate in liver veal agar or nutrient agar plate to conduct anaerobic cultivation for them; pick anaerobic cultures from pure cultivation and inoculate in liver veal agar or nutrient agar plate to conduct aerobic cultivation for them, to identify whether the cultures are facultative anaerobes or not.

B.2.7 If testing the botulinum toxin of clostridium is required, typical colonies should be selected for pure culture

in cooked meat medium. Cultivate at 36℃ for 5 days. Then conduct botulinum toxin test in accordance with GB/T 4789.12.

B.3 The Inoculation and Culture of Acid Canned Food (pH≤4.6)

B.3.1 Inoculate each sample in four test tubes of acid broth and two malt extract broth. Inoculate 1mL(g)~2mL(g) of samples (1 mL~2 mL for liquid samples, 1g~2g for solid samples, if both exist, half of each for both) in each test tube. The conditions of culture are shown in Table B.2.

Table B.2 The Acid Broth and Malt Extract Broth for Inoculation of Acid Canned Food (pH≤4.6)

Culture Medium	Number of Tubes	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.3.2 After culturing in accordance with the conditions shown in Table B.2, record states of microbial growth in each tube. Discard it after recording if no microbial growth is observed in a tube.

B.3.3 If there is microbial growth in a culture tube, pick the cultured content to make a smear and conduct Gram staining microscopy for it and record the microorganisms observed.

B.3.4 If microbial growth is found in acid broth or malt extract broth under the culture condition of 30℃, inoculate the contents of each positive tube respectively in 2 nutrient agar or Sabouraud dextrose agar plates, one for aerobic cultivation, the other for anaerobic cultivation.

B.3.5 If microbial growth is found in acid broth under the culture condition of 55℃, inoculate the contents of each positive tubes respectively in 2 nutrient agar or Sabouraud dextrose agar plates, one for aerobic cultivation, the other for anaerobic cultivation. Conduct smear staining microscopy for the plate where there is microbial growth and report the microbial types observed in microscopy. The cultivation procedures are shown in Figure B.2.

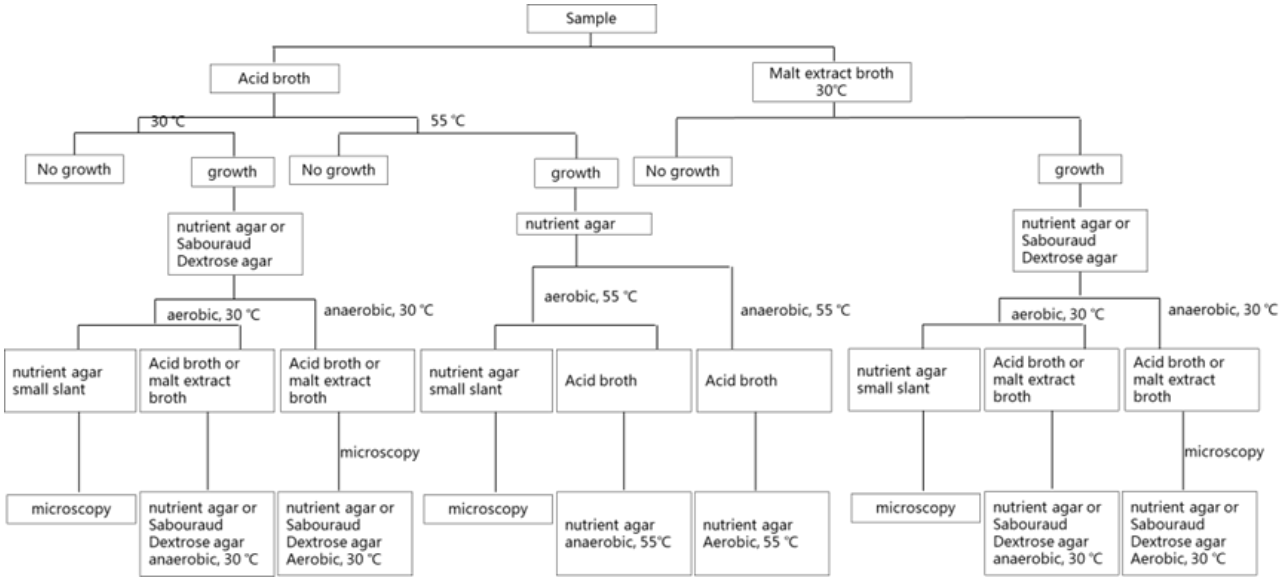

Figure B.2 The Procedures of Inoculation and Culture of Acid Canned Food

B.3.6 Pick single colonies from the nutrient agar or sabouraud dextrose agar plates aerobically cultured at 30℃ and inoculate them in the nutrient agar small slant, which will be used for the later Gram's staining microscopy. Meanwhile, inoculate in acid broth or malt extract broth and perform pure culture.

Pick single colonies from the nutrient agar or sabouraud dextrose agar plates anaerobically cultured at 30℃ and inoculate them in acid broth or malt extract broth and perform pure culture.

Pick single colonies from the nutrient agar plates aerobically cultured at 55℃ and inoculate them in nutrient agar small slant, which will be used for the later Gram' s staining microscopy. Meanwhile, inoculate in acid broth for pure culture.

Pick single colonies from the nutrient agar plates anaerobically cultured at 55℃ and inoculate them in acid broth for pure culture.

B.3.7 Pick cultures from nutrient agar small slant to make a smear and take a look under a microscope. Pick cultures from acid broth or malt extract anaerobically cultured at 30℃ and from acid broth anaerobically cultured at 55℃, respectively make a smear for them and take a look under a microscope.

B.3.8 Inoculate the pure cultures aerobically cultured at 30℃ in nutrient agar or sabouraud dextrose agar plate and conduct anaerobic culture. Inoculate the pure cultures anaerobically cultured at 30℃ in nutrient agar or sabouraud dextrose agar plate and conduct aerobic culture. Inoculate the pure cultures aerobically cultured at 55 ℃ in nutrient agar and conduct anaerobic culture. Inoculate the pure cultures anaerobically cultured at 30℃ in nutrient agar and conduct aerobic culture. Each step is for identifying whether the pure cultures belong to facultative anaerobes.

B.3.9 Results Analysis

B.3.9.1 If the growth of microorganisms cannot be found in inflated samples, the expansion may be caused by the hydrogen which is generated by the reaction between the content and packaging. The amount of generated hydrogen varies with the length of storage time and conditions as well. Filling excessive contents in packages may also give rise to slight expansion, which can be determined by weighing.

If the mixture of a large number of microorganism is observed in direct smear examination and these microorganisms cannot grow after culture, it indicates that there is putrefaction before sterilization. The pH, smell and morphological structure of products are found aberrant due to the microbial growth before seal packing.

B.3.9.2 Only the growth of bacilli found under the culture condition of 36℃ with packaging containers perfectly sealed and the heat resistant performance of such bacilli are not better than that of clostridium botulinum, which indicates that under-sterilization occurs during the production process.

B.3.9.3 If the mixed colonies of bacilli, cocci and fungi appear after culture, it indicates there is a leakage in packaging containers. It might result from under-sterilization as well, but in this condition, the expansion rate of the same batch of products would be rather high.

B.3.9.4 Observe the situations of acid production and gas production in bromcresol purple dextrose broth

cultured at 36℃or 55℃. If acid is produced, it indicates the growth of mesophilic microorganisms (e.g. mesophilic acid-resisting bacteria) or thermophilic microorganisms (e.g. Bacillus stearothermophilus).

Bacteria growth and gas with putrid odour production in cooked meat medium at 55℃ indicate that the putrefaction of samples results from thermophilic anaerobic clostridium.

Bacteria growth and gas with putrid odour production in cooked meat medium at 36℃ and spores observed in microscopy indicate that the putrefaction may be caused by clostridium botulinum, clostridium sporogenes and clostridium perfringens. Botulinum toxin can be further tested if necessary.

B.3.9.5 The spoilage of acid canned food is usually caused by lactobacillus with no spores and yeast.

Generally, if its pH is lower than 4.6, the deterioration caused by bacillus does not happen. The spoiled ketchup or canned tomato sauce wouldn't be expanded, but putrid odor with or without pH getting low may occur. It is usually caused by aerobic bacillus.

B.3.9.6 The thermophilic bacteria exist in many canned foods. They don't grow in normal storage conditions, but they will grow and result in putrefaction when the products are exposed to high temperature (50℃~55℃). Thermophilic acid-resisting bacillus and bacillus stearothermophilus may cause the putrefaction respectively in acid and low-acid food, but there wouldn't be expansion in the packaging containers. Culture at 55℃ will not give rise to the change of the appearance of packaging containers, yet producing the putrid odor with and without lower pH. The spoilage of tomatoes, pears, figs and pineapples is sometimes caused by clostridium pasteurianum. As a kind of thermophilic anaerobic bacteria, Clostridium thermosaccharolyticum can cause the expansion and products' putrid odor.

Thermophilic anaerobic bacteria can produce the gas as well, because it proliferates quickly after beginning to grow. It may be confusing whether the expansion is caused by hydrogen or the gas produced by thermophilic anaerobic bacteria. Chemical decomposition generates carbon dioxide, which commonly occurs especially in sugary and sour food, for example, tomato sauce, molasses, mincemeat and sugary canned fruits. The decomposition speeds up with the temperature increasing.

B.3.9.7 Laboratory pollution should be considered if any microorganism is isolated from the direct smears of sterile vacuum package and normal products. To confirm whether it is lab pollution, inoculate the isolated living microorganism under aseptic conditions to another normal control sample, then seal and culture at 36 ℃ for 14 days. It is likely that these microorganisms are not from the original samples if expansion or the deterioration of products occurs. Open the packages of samples under aseptic conditions and re-culture as per the procedures mentioned above if the samples are still flat. If the same kind of microorganism is found again and the products are normal, the products would be considered commercially sterile because this kind of microorganisms wouldn't grow during the normal storage and transportation.

B.3.9.8 Determinacy conclusions cannot be made through broth culture if there is corruption in the food itself. In this situation, further culture is necessary to confirm whether there is growth of microorganism.

B.4 Leakproofness Test Method for Empty Food Can Made of Tinned Steel Sheet

B.4.1 Pressure-reduce Leakproofness Test

Wash the packaging cans used as samples well, and dry it at the temperature of 36℃. Fill the dried cans with clean water accounting for 80%~90% of each can. Place the perspex sheet with a rubber ring onto the open curled end of cans to keep it sealed. Start vacuum pump and close the gate for releasing gas. Press the cover board with

hands and control the air bleed. Make sure that the vacuum gauge rising from 0 Pa to 6.8×104Pa (510mmHg) lasts more than 1 minute and the vacuum degree of 6.8×10^4Pa(510mmHg) lasts more than 1 minute. Tilt the can and carefully observe it in order to see whether there is bubble generated, especially the hemming and commissure. It can be judged to be leaky if the bubbles are constantly produced at the same site. Record the time of leakage and vacuum degree and mark the site where there is leakage.

B.4.2 Pressure-increase Leakproofness Test

Clean the can used as sample and dry it under the temperature of 36℃. Plug the mouth of the empty can with a rubber stoppers, soak the empty can in the glass jar full of water, start the air compressor and slowly open the valves, to gradually increase the pressure in the can to $6.8\ x10^4$ Pa and keep the pressure for two minutes. Careful observation of the can should be made, the hemming and commissure in particular, to see if bubbles are produced. It can be judged to be leaky if the bubbles are constantly produced at the same site. Record the time of leakage and pressure, and mark the site where there is leakage.