HPB Method **MFHPB-01**
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HEALTH PRODUCTS AND FOOD BRANCH

OTTAWA

DETERMINATION OF COMMERCIAL STERILITY AND THE PRESENCE OF VIABLE MICROORGANISMS IN CANNED FOODS

1. APPLICATION

This method is applicable to the detection of viable microorganisms in commercially sterile foods packaged in hermetically sealed containers to determine compliance with the requirements of Division 27 and Sections 4 and 7 of the Food and Drugs Act and pertinent sections of the Canada Agricultural Products Act, the Meat Inspection Act and the Fish Inspection Act. This method replaces MFHPB-25C dated March 1989.

2. PRINCIPLE

This method employs the aseptic sampling of the contents of hermetically sealed foods and the subsequent inoculation of suitable media to determine the presence or absence of viable organisms.

A portion of the product is mixed with specified media and incubated under specified conditions of time and temperature. It is assumed that each viable microorganism will multiply under these conditions and give rise to a visible colony in solid media or turbidity in liquid media.

This method is also used to characterize such organisms as follows:

- as being present in mixed or pure populations;
- as being anaerobic, aerobic or facultative;
- as being mesophilic or thermophilic;
- as having coccal, bacillal or spiral morphology.

3. DEFINITION OF TERMS

3.1 See Appendix A of Volume 2.

3.2 Commercial Sterility: the condition achieved by the application of heat, alone or in combination with other treatments, to render a food free from viable forms of microorganisms, including spores, capable of growing in the food at temperatures at which the food is designed normally to be held during distribution and storage. If the normal temperature for storage or handling of the product is higher than 40°C then analyse for Thermophiles. Otherwise, analyse for Mesophiles only.

This definition does not apply to low acid foods which are to be kept under refrigeration or frozen and labelled as such or to tomato products which have a pH of 4.7 or less after heat processing.

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3.3 Hermetically Sealed Containers: containers designed and intended to be secure against the entry of microorganisms, including spores. These containers include metal cans, glass jars, flexible packages such as retort pouches and Tetrapaks, etc.

3.4 Low Acid Food: a food, other than alcoholic beverages, where any component of the food has a pH greater than 4.6 and a water activity greater than 0.85.

3.5 Acid and Acidified Food: a food with a pH equal to or lower than 4.6.

3.6 Water Activity: the ratio of the vapour pressure of a food to the vapour pressure of pure water, at the same temperature and pressure.

3.7 Refrigeration: exposure to a temperature of 4°C or less but not frozen.

3.8 Semi-Liquid Products: products that may pour with difficulty, but can be mixed by shaking the container. Prior to opening containers containing liquid products, the contents should be mixed by shaking the unopened container.

3.9 Semi-Solid Products: products that have a high viscosity; they pour with great difficulty, and cannot be mixed by shaking the unopened container.

4. COLLECTION OF SAMPLES

4.1 See Appendix B of Volume 2.

4.2 During storage and transport of open containers, swollen or leaking containers and suspect food poisoning samples, keep the sample units properly contained (i.e., in sealable plastic bags placed in water-tight metal or plastic bins) and refrigerated. Whenever possible, the contents of the open containers can be aseptically transferred to sterile containers.

4.3 For aseptically processed and packaged products: each container that has been collected earlier than 30 days after packaging should be pre-incubated for 7 days at 30-35°C°C before the analytical unit is taken for analysis. However, this procedure may not apply to situations such as illness investigations or evident lack of commercial sterility.

5. MATERIALS AND SPECIAL EQUIPMENT

1) Laminar air flow cabinet or biological safety cabinet, located in a clean room

Carry out all microbiological analyses under a laminar flow cabinet. The particle count of the laminar flow should not exceed a total of 100 particles of size 0.5 micron or greater per cubic foot, (Class 100, U.S. Federal Standard 209B). A Class 10,000 clean room is acceptable under the provisions detailed in section 8.1.3. A class 100,000 clean room has also proved to be satisfactory providing additional quality assurance measures as detailed in section 8.1.3 are taken.

2) Sterile gowns, caps, gloves and face masks or equivalent. Face masks are optional.

Minimum acceptable protective clothing will be: clean laboratory smock with protective sleeves and tight fitting cuffs; surgical gloves; cap to cover hair; mask covering mouth, nose and facial hair. Gloved hands shall be disinfected as required.

3) Sanitary can openers (such as Bacti-Disc Cutters, Wilkens-Anderson Co.) or other sterile devices to open the containers

4) Sterile screw-cap culture tubes (20 X 150 mm)

5) Sterile cotton-plugged transfer pipettes (for liquid and semi-liquid products)

6) Sterile spoons, spatulas, probes, plier, cork borers, etc (for viscous, semi-solid and solid products)

7) Sterile loops

8) Anaerobe jars

9) Sanitizing solution, such as, chlorine

10) Plastic container of 2 to 10 L capacity for soaking containers in the sanitizing solution

11) Chlorine-indicating tape (Diversey Wyandotte) or equivalent

12) 70% alcohol (optional)

13) pH meter or paper capable of distinguishing 0.3 to 0.5 pH within a range of 6.0 to 7.5

14) Sterile, sealable containers to store remainder of food samples for further tests

15) Gram stain solutions

16) Light microscope

17) Control strains; use the following or equivalent strains:

anaerobic mesophilic sporeformer: *Clostridium sporogenes* ATCC 7955 (NFPA 3679) or *C. histolyticum*
aerobic growth (30°C): *Bacillus coagulans* ATCC 8038 (NFPA 43P)
aerobic obligate thermophilic growth: *Bacillus stearothermophilus* ATCC 12016 (NFPA 2184)
obligate anaerobic thermophilic growth (55°C): *Clostridium thermosaccharolyticum* ATCC 7956

18) Diamond point pen or other marking device

19) Sterile examination trays (Pyrex or enamel baking pans)

20) Sterile plastic bags (for handling of swollen containers as described in 4.1 and 7.3.2) or equivalent containment devices

21) Incubators, 30, 35 and 55°C

22) Waterbaths, 80^0, 100^0C

NOTE: It is the responsibility of each laboratory to ensure that the temperature of the incubators or water baths are maintained at the recommended temperatures. Where 35°C is recommended in the text of the method, the incubator may be at 35 +/-1.0° C. Similarly, lower temperatures of 30 or 25 may be +/- 1.0°C. However, where higher temperatures are recommended, such as 43 or 45.5°C, it is imperative that the incubators or water baths be maintained within 0.5°C due to potential lethality of higher temperatures on the microorganism being isolated.

23) Media and reagents

Note: Most of the media listed below are commercially available and are to be prepared and sterilized according to the manufacture's instructions.

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LOW ACID FOODS

Note: For shelf stable milk and milk products: to aid in the recovery of microorganisms, add 1 mL of resterilised product to each litre of tempered media before pouring the plates.

Enrichment

PE-2
Cooked Meat Media (CMM)

Plating

Plate Count Agar (PCA)
Tryptic Soy Agar (TSA)
Blood Agar (BA)
Reinforced Clostridial Media (RCM)
Liver Veal Agar (LVA)

Optional

Baird-Parker Agar
MacConkey's Agar

ACID AND ACIDIFIED LOW ACID FOODS

Note: The pH of the media (both broths and agars) to be used in analysing an acid or acidified food may have to be adjusted to the pH of that food. Prior knowledge of the pH of the food to be analysed may be sufficient. However in cases in which the pH of the food to be analysed is unknown, the pH of the contents of at least one can must be determined.

Enrichment

Acid Broth (AB)

Plating

Potato dextrose Agar (PDA)
Sabouraud Dextrose Agar (SDA)
Thermoacidurans Agar (TA)
Orange Serum Agar (OSA); Filter Aid is needed for OSA

6. SAFETY PRECAUTIONS

HANDLE ALL SWOLLEN CONTAINERS AS IF THEY CONTAINED *CLOSTRIDIUM BOTULINUM* AND/OR THEIR TOXINS UNTIL THE ABSENCE OF SUCH HAS BEEN ESTABLISHED. NEVER TASTE FOOD FROM A CONTAINER UNDER INVESTIGATION. ALWAYS USE MECHANICAL PIPETTING DEVICES.

SWOLLEN CONTAINERS, ESPECIALLY HARD SWELLS, CAN SPRAY THEIR CONTENTS UPON BEING PUNCTURED. TO PREVENT CONTAMINATION OF SURROUNDING ENVIRONMENT, SUITABLE HANDLING PRACTICES MUST BE USED. IT IS RECOMMENDED TO HANDLED SWOLLEN CONTAINERS IN A CLASS II BIOSAFETY CABINET WHICH PROVIDES PROTECTION TO THE OPERATOR AS WELL AS THE PRODUCT.

FOLLOW ALL PRECAUTIONS AND METHODOLOGY FOR ANALYSIS PERTAINING TO *C. BOTULINUM* AS DESCRIBED IN MFHPB-16.

7. PROCEDURE

Analyse each sample unit individually. The test shall be carried out in accordance with the following instructions:

7.1 Handling and preparation of the sample unit

7.1.1 Identification of sample unit

Legibly identify each sample unit directly on the container using a diamond pen point or other device. This must be done in a manner so that the identity is not lost or removed during subsequent handling and testing. Ensure that the identity relates to the sample from which each sample unit is derived. The complete code shall be recorded for each sample unit.

7.1.2 Removal and identification of the label

Place coincident marks on the label and the container. The marks on the container could be made with indelible ink, carborundum or diamond point pen so that they will not be removed during subsequent handling of the container. The intent is to permit establishment of the position of the label on the container after its removal. Carefully remove the label so as to keep it as intact as possible (not applicable to lithographed cans and printed flexible packages). Mark the sample and sample unit numbers on the label.

7.2 Cleaning and disinfection of external container surface and equipment

Scrub the containers with detergent and water, if necessary. Prepare sufficient volume of chlorine solution to allow for complete immersion of the containers to be disinfected. Completely immerse the containers in the chlorine solution or flood the end to be opened with the solution for a minimum immersion time of 20 minutes. Periodically verify the concentration of the active (free) chlorine. Chlorine test papers may be used for this purpose. (This solution remains effective until the concentration falls below 100 ppm of available chlorine; at this point, prepare fresh solution.) Containers must be removed by using suitable sterile instruments, e.g. tongs, gloves. Alternatively, the external container surface can be decontaminated by flooding or spreading with a 2% solution of peracetic acid in an appropriate wetting agent (e.g., 0.1% polysorbitan 80) for 5 minutes. Appropriate safety precautions should be taken when using any of these chemical disinfectants.

After disinfection, place the containers with the end to be opened facing up (usually the non coded end) on the previously disinfected surface under a properly operating laminar air flow hood. Before piercing the container, ensure that the surface to be opened is completely dry. Allow the containers to air dry under laminar flow or dry the containers with sterile disposable paper tissues or towels.

7.3 Opening the container

7.3.1 Normal containers (non-swollen)

For liquid or semi-liquid products: thoroughly mix the contents before sampling. This can be accomplished by rotating the container end over end at least 20 to 30 times just prior to opening. Place the container with the coded end down.

Use a sterile or disinfected opener for each container. Other sterile piercing device may also be used. To open the container cut a hole in the can end to ¼ inch (6.35 mm) from the double seam to permit adequate sampling of the contents and to facilitate the micro-leak vacuum test.

To disinfect an opener after use, and for immediate re-use, remove any adhering product, immerse the complete tip in a 70% aqueous solution of ethanol and flame the entire end to include both piercing and cutting edges. Before re-use allow it to cool under the laminar flow hood. Disinfection can also be accomplished by immersion in the disinfection solution (see section 7.2) for at least 20 minutes. After removal allow to air dry under the laminar flow hood or dry by flaming.

7.3.2 Swollen containers

Note: See Section 6 (Safety Precautions) before proceeding.

Place the container in a sterile plastic bag and while holding the open end of the bag firmly about the opener shaft, pierce the container. Do not remove the container until it has completely vented. This method has in practice proven to be the best for swollen flexible pouch products.

After completion of venting, use a sterile opener to aseptically open the container by cutting a hole in the can end to ¼ inch (6.35 mm) from the doubleseam to permit adequate sampling of the contents and to facilitate the micro-leak vacuum test.

7.4 Sampling container contents

Immediately before inoculating the media, aseptically transfer a portion of at least 50 g of the food to a sealable, sterile container. Label and keep under refrigeration in a manner to prevent subsequent contamination of the product. Remove one to two millilitres or grams of product from the container for inoculation of each of the required liquid media. If direct plating is performed, each solid media plate should be streaked with at least one loopful of the contents (approximately 0.01 mL). Pour plates could also be used.

7.4.1 Liquid and semi-liquid products

Remove the appropriate quantity using suitable sterile pipettes or other sterile devices.

7.4.2 Solid and semi-solid products

Viable microorganisms may be localized in solid and semi-solid products. They are more likely to be in the centre of the product if under processing has occurred, or near the seams or closure if the container has leaked.

Samples from the centre can be obtained by taking a core from the contents using a tool such as a sterilized cork borer. To adequately sample the external surface of solid products, the entire contents, if possible, should be removed from the can to a sterile tray. This may require the removal of the entire end of the can by a sterilized conventional can opener. If the analyst

is planning to do a vacuum leak detection on this sample unit, then this test should be completed prior to removing the end of the can.

If both doubleseams are suspect and further tests to evaluate their integrity are anticipated, alternate aseptic means of opening the can to permit the removal of the entire contents will have to be used. In some instances scored cans can be opened using the key or similar device. If the key is used, check if there is any damage in the score area before using it.

Scrape the external product surface, especially the area in close proximity to the seams, with a sterile spatula, to obtain the analytical portion. In most cases both the product center and the external product surface must be sampled.

7.5 Microbiological analysis of the content

For the flow charts for the microbiological analyses, see Diagrams.

To determine Commercial Sterility:

If the normal temperature for storage or handling of the product is higher than 40°C the product should be analysed for thermophiles. Otherwise, proceed with "isolation of Mesophiles" only.

For other samples:

Samples, such as those from underprocessed lots, involved in food poisonings, or from cans which are visibly swollen, should be analysed for both Mesophiles and Thermophiles.

Controls See 8.2 for the use of negative food product controls and negative and positive media controls. Controls should be used throughout the following analysis.

7.5.1 **ISOLATION OF MESOPHILES**:

Note: A specific temperature between 30 and 35°C may be used as long as the temperature chosen does not vary more than ± 1.0°C.

7.5.1.1 **LOW ACID FOODS** (see flow diagram)

Enrichment:

Inoculate 2 tubes of sterile PE-2 and 2 tubes of sterile CMM. Include positive and negative media controls. See sections 8.2.1 and 8.2.2. Incubate tubes aerobically at 30 to 35°C for at least 14 days. Examine the tubes for growth daily. Prepare duplicate streak PCA, TSA or BA plates from all positive and suspected positive tubes. Incubate one plate aerobically and one plate anaerobically at 35°C for 2 to 5 days and examine daily. For anaerobes, RCM or LVA incubated anaerobically, may be used. Identify the Gram reaction and the cell morphology for each colony type observed on the plates.

Direct plating (see flow diagram)

Generally PCA, TSA or BA plates are used; however for anaerobes, RCM or LVA media may be used. In cases in which the presence of specific microorganisms are suspected, then corresponding media, e.g. Baird-Parker, MacConkey's etc., should be used.

Streak or pour two PCA plates (RCM or LVA plates may be used for anaerobes) and incubate one plate aerobically and one plate anaerobically at 35°C for 2 to 5 days. Observe the colony types on each plate. Obtain Gram reaction and cell morphology for each colony type.

7.5.1.2 **ACID AND ACIDIFIED LOW ACID FOODS** (see flow diagram)

Enrichment:

Inoculate 2 tubes of sterile AB medium. Alternatively or additionally, when the product is fluid or semi-fluid, the normal food product itself, after re-sterilization, may be used as the growth medium. (If the food product is solid you may have to add recently boiled distilled water to get the right consistency).

Incubate 1 set of tubes aerobically and the other anaerobically at 30 to 35°C for at least 14 days. Examine the tubes for growth after 3, 7 and 14 days. (Because it is frequently difficult to observe growth in food products used as a medium, direct smears and streak plates should be used to determine growth). Prepare duplicate streak PDA plates and duplicate pour plates of TA.

Incubate plates aerobically and anaerobically at 30°C for 2 to 5 days and examine daily. Identify the Gram reaction and the cell morphology for each colony observed on the plates.

Direct plating (see flow diagram)

Streak or pour two PDA plates and two TA plates and incubate one plate aerobically and one plate anaerobically at 35°C for 2 to 5 days. Observe the colony types on each plate. Determine Gram reaction and cell morphology for each colony type. Inoculate other appropriate media in cases where the presence of specific organisms is suspected.

7.5.2 **ISOLATION OF THERMOPHILES:**

7.5.2.1 **LOW ACID FOODS** (see flow diagram)

Enrichment:

Inoculate 2 tubes of sterile PE-2 and 2 tubes of sterile CMM. Heat-shock one tube of each type of the inoculated media for 10 minutes at 80°C (ensure that the media has reached 80°C prior to counting the 10 minutes). Incubate all tubes at 55°C for a maximum of 7 days and examine daily. Treat positive and suspected positive tubes as described in 7.5.1.1 above, except incubate plates at 55°C.

Direct plating

Note: For shelf stable milk and milk products: to aid in the recovery of microorganisms, add 1 mL of resterilised product to each litre of tempered media before pouring the plates.

Streak or pour two PCA plates (two RCM or LVA plates may be used for anaerobes) and incubate one plate aerobically and one plate anaerobically at 55°C for 2 to 5 days. Observe the colony types on each plate. Obtain Gram reaction and cell morphology for each colony type.

7.5.2.2 **ACID AND ACIDIFIED LOW ACID FOODS** (see flow diagram)

Enrichment:

Inoculate 2 tubes of sterile AB medium or 2 tubes of medium made from re-sterilized normal food product (as described above). Proceed as detailed above for thermophiles.

Direct plating

Streak or pour two PDA plates aerobically and two TA plates anaerobically and incubate one plate aerobically and one plate anaerobically at 55°C for 2 to 5 days. Observe the colony types on each plate. Obtain Gram reaction and cell morphology for each colony type. Inoculate other appropriate media in cases where the presence of specific organisms is suspected.

7.6 Retained sample units

The retained sample units may be used for toxin analysis, (*C. botulinum*: see MFHPB-16; *S. aureus*: see MFLP-47; etc.), chemical analysis (for example pH) or for a repetition of the microbiological analysis in order to validate results. However, refrigeration temperatures can affect bacterial densities, and results obtained from this sample unit must be interpreted with care.

7.7 Direct Microscopic Examination (DME) of the Product (see MFHPB-02)

Frequently, evidence of pre- or post-processing microbial growth can be obtained by DME of the product. Although this test does not require aseptic conditions, smears for examination should be prepared after sampling of the container content.

7.8 Determination of pH (see MFHPB-03)

Microbiological growth is frequently accompanied by a significant change in pH. Therefore, the pH of the sampled food may provide evidence of growth and should be measured as soon as possible following the microbiological sampling.

7.9 Organoleptic examination

Examine the product for off-odours or other evidence of spoilage, e.g., frothing, curdling, discolouration, etc. DO NOT TASTE THE PRODUCT. Record observations.

7.10 Identification of microorganisms

While detailed identification of viable microorganisms is not required to establish conformity with the Act and Regulations in routine sampling, it is necessary in specific cases (compliance action, recalls, etc.) to identify the isolates obtained from plating of positive tubes. Such needs will be dictated by circumstances encountered in each case. In the cases of product recall and in compliance activity, it

may be necessary to identify all microorganisms. In some cases, it may be necessary to send the cultures to a Reference Laboratory.

7.11 Disposal

Prior to disposal, autoclave the container and the contents after sampling if there is reason to believe that the contents are not commercially sterile. If *C. botulinum* is suspected to be present, then the container and contents must be autoclaved before disposal.

8. QUALITY CONTROL PROCEDURES

While normal microbiological laboratory quality control procedures are applicable, because of the nature of this method, specific attention must be given to the following:

8.1 Laminar flow hood and clean rooms

8.1.1 Laminar flow hood

Carry out a DiOctyl Pthalate (DOP) challenge test or an equivalent test, and measure the air velocity at least annually, and more frequently as required. Record the results of the tests.

8.1.2 Biological safety cabinet

If a biological safety cabinet is used, the cabinet must be certified at least annually and the results of the certification tests recorded.

8.1.3 Clean rooms (Class 10,000 or 100,000)

Regularly check the microbiological quality of the air in the rooms using a Lusella slit-type air sampler (or other type of air sampler) and/or exposure of open plates for one hour. Record results of all tests.

For Class 10,000 clean rooms, this should be carried out at least once during each operation or every week if the room is in continuous operation.

For Class 100,000 rooms, this should be carried out continuously during every test operation.

8.2 Controls

8.2.1 Negative Food Control

If deemed necessary, a negative food control can be prepared. The negative food control is prepared from a sample unit or an aliquot from the product that has been sterilized in an autoclave at 121°C for 15 min. Inoculate 2 tubes of PE-2 and 2 tubes of CMM with an aliquot of the sterile food.

Note: A negative food control may be useful in the interpretation of results where it is suspected that the data will not provide a clear-cut answer as to the commercial sterility of a product or where previous results with the same product have provided conflicting results.

8.2.2 Media Controls

Negative control

Pre-incubate media for a minimum of two days, or incubate one unit of medium per sample for each batch of medium used. Record all results.

Positive control

Inoculate one unit per batch using appropriate organisms for conditions of the medium composition and temperature of incubation as listed below. Record all results.

	Organism	Media	Incubation Temperature
1.	*Clostridium sporogenes* or *C. histolyticum*	PE2, CMM, LVA	35°C
2.	*Bacillus coagulans*	Thermoacidurans Agar, Acid broth, PDA	30°C
3.	*B. stearothermophilus*	PE2, CMM, PCA	55°C
4.	*C. thermosaccharolyticum*	PE2, CMM, LVA	55°C

9. REFERENCES

The analyst is referred to the following or other suitable references:

9.1 American Public Health Association (APHA). 1992. Compendium of Methods for the Microbiological Examination of Foods, 3rd edition, American Public Health Association, Washington, D.C.

9.2 Anon. 1994. Bergey's Manual of Determinative Bacteriology. 9th edition. W.R. Hensyl (editor). The Williams and Wilkins Co., Baltimore.

9.3 Anon. 1973. Federal Standard (U.S.) 209B: Clean Room and Work Station Requirements, Controlled Environment.

9.4 Association of Official Analytical Chemists (AOAC). 1995. Chapter 21. Bacteriological Analytical Manual, Arlington, Virginia.

9.5 Atlas, R.M. 1997. Handbook of Microbiological Media, 2nd edition, L.C. Parks (editor). CRC Press Inc.

9.6 Austin, P.R. 1970. Design and Operation of Clean Rooms. Revised Edition. Business News Publishing Company, Birmingham, Michigan.

9.7 Health Canada. 1996. Laboratory Biosafety Guidelines, 2nd edition, Laboratory Centre for Disease Control, Health Canada.

10. PREPARATION OF MEDIA

For steam sterilization, it is essential that the load be sufficiently pre-heated before the actual sterilization period commences. This varies considerably with the nature and size of the load. Hence, proper exposure times should be followed to ensure sterilization of flask solutions and heat stable culture media, particularly when prepared in large volumes (Refer to your sterilizer manual).

10.1 Acid Broth (AB)

Proteose peptone	5 g
Yeast extract	5 g
Dextrose	5 g
K_2HPO_4	4 g
Distilled H_2O	1000 mL

Adjust to pH of the medium to the pH of the food analysed with 1N HCl solution. Sterilize at 121°C for 20 minutes or equivalent.

10.2 Cooked Meat Medium (CMM)

Beef heart	454 g
Proteose peptone	20 g
Dextrose	2 g
Sodium chloride	5 g

The medium may be prepared by distributing 2.5 g of a prepared and dehydrated medium into 20 X 150 mm screw-cap culture tubes, adding 20 mL of cold distilled water, and mixing thoroughly, letting it stand to ensure thorough wetting of all particles. Sterilize for 15 minutes at 121°C or the equivalent. Final pH should be 7.2. Normally the prepared sterilized media can be maintained unaffected under refrigeration (0°C to 4°C) for up to 4 weeks. Prior to use, the refrigerated medium should be deaerated at 100°C for 10 minutes and cooled before use.

10.3 Disinfecting solution for containers - chlorine

Stock buffer solution (1.0 Molar solution)

Potassium Dihydrogen Phosphate (KH_2PO_4)	136 g
Distilled water	1000 mL

Adjust to pH 6.2 with 1N NaOH solution.

Preparation of a buffered chlorine disinfecting solution
Dilute the stock buffer solution 20 fold, (e.g., 5 mL buffer solution made up to 100 mL with distilled water). If the diluted stock solution is going to be held longer than one working day prior to use, it should be sterilized at 121°C for 15 minutes in suitable bottles.

Prepare the chlorine solution by adding 10 mL of a commercial chlorine solution (e.g. Javex at 5.25% available chlorine or other suitable chlorine solution of comparable strength) to each litre of the diluted buffer (1/20M) solution. The final available chlorine concentration should be between 500 and 525 ppm and the final pH 6.5. The available chlorine should not be less than 100 ppm.

10.4 Liver Veal Agar (LVA)

Liver, infusion from	50 g
Veal, infusion from	500 g
Proteose peptone	20 g
Neopeptone	1.3 g
Tryptone	1.3 g
Dextrose	5.0 g
Soluble starch	10.0 g
Isoelectric casein	2.0 g
Sodium chloride	5.0 g
Sodium nitrate	2.0 g
Agar	15.0 g
Distilled water	1000 mL

Mix ingredients in distilled water. Sterilize at 121°C for 15 minutes.

Final pH should be 7.3 ± 0.3.

10.5 Modified PE-2 Medium (PE2)

Basal media:

Peptone	20 g
Yeast extract	3 g
2% Alcoholic solution of bromocresol purple	2 mL
Distilled water	1000 mL
Alaska seed peas	8-10/tube

Dissolve ingredients with gentle heating, if necessary, and dispense 19 mL portions into 20 x 150 mm screw-cap culture tubes containing 8-10 untreated Alaska seed peas. (The peas may be obtained from W.H. Perron Seed Company, 515 La Belle Blvd., Laval, P.Q. or other suppliers). Sterilize for 30 minutes at 121°C or an equivalent. Normally the prepared sterilized media can be maintained unaffected under refrigeration (0°C to 4°C) for up to 4 weeks. Prior to use, the refrigerated medium should be deaerated at 100°C for 10 minutes and cooled before use. Cool at ambient temperature prior to use.

10.6 Orange Serum Agar (OSA)

Tryptone or trypticase	10.0 g
Yeast extract	3.0 g
Dextrose	4.0 g
Dipotassium phosphate	2.5 g
Agar	17.0 g
Cysteine	0.001 g
Orange serum	200 mL
Distilled water	800 mL

Dissolved ingredients in distilled water. Prepare orange serum by heating 1 litre of freshly extracted orange juice or reconstituted frozen orange juice concentrate to approximately 93°C (200°F). Add 30g of "filter aid" and mix thoroughly. Filter under suction through a Buchner funnel using coarse filter paper precoated with "filter aid". Discard the first few mL of filtered serum.

Sterilize 15 minutes at 121°C. After sterilization, the pH should be about 5.5. Do not over autoclave.

10.7 Plate Count Agar (PCA)

Tryptone	5 g
Glucose	1 g
Yeast extract	2.5 g
Agar	15 g

Add ingredients to 1 litre of distilled water, heat to boiling with constant stirring to obtain complete solution of all ingredients. Cool to 45°C to 60°C, and adjust the pH of the solution so that after autoclaving it will be 7.0 ± 0.1. Dispense as required, and sterilize by autoclaving at 121°C for 15 minutes or its equivalent. For shelf stable milk products, addition of 1 mL of resterilised product, to the tempered media before pouring the plates, may aid in recovery of microorganisms.

10.8 Potato Dextrose Agar (Acidified) (PDA)

Basal media:

Infusion from white potatoes	200 mL
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 mL
10% Tartaric acid	16.0 mL

Suspend 39 grams of commercial dehydrated ingredients in distilled water. Heat mixture to boiling to dissolve ingredients. Autoclave 15 min. at 121°C (15 lb pressure) and cool to 45-50°C. Lower the pH to 3.5 ± 0.1 by the addition of 16 ml of sterile 10% tartaric acid and dispense into Petri plates. Do not reheat the acidified medium as this will hydrolyse the agar.

10.9 Reinforced Clostridial Medium (RCM)

Yeast extract	3 g
Beef extract	10 g
Peptone	10 g
Soluble starch	1 g
Dextrose	5 g
Cysteine hydrochloride	0.5 g
Sodium acetate, anhydrous	3 g
Sodium chloride	5 g
Agar (for solid medium)	15 g
Distilled water	1000 mL

Heat to boiling to dissolve ingredients and adjust pH to 7.4. Sterilize by autoclaving at 121°C for 15 minutes or an equivalent.

10.10 Sabouraud Dextrose Agar (SDA)

Dextrose	40.0 g
Peptone	10.0 g
Agar	15.0 g
Distilled water	1000 mL

Dissolve ingredients in distilled water and heat to boiling; dispense in flasks; sterilize in autoclave at 121°C for 15 minutes; pH 5.6 ± 0.2. Do not over autoclave.

10.11 Thermoacidurans Agar (TA)

Yeast extract	5.0 g
Proteose peptone	5.0 g
Dextrose	5.0 g
Dipotassium phosphate	4.0 g
Agar	20.0 g
Distilled water	1000 mL

Suspend ingredients in distilled water and dissolve agar by boiling. Distribute into flasks or bottles. Autoclave 15 minutes at 121°C. Final pH should be 5.0.

Diagram 1. Microbiological Analysis of the Contents of Canned Foods that are Low Acid using Liquid Aerobic/Anaerobic Media.

Swollen Container
(Use containment devices. Handle as if it contained *C. botulinum*)
or
Non-swollen container

↓

Cleaning and Disinfection

↓

Liquid Aerobic/Anaerobic Media

↙ ↘

Mesophiles

2 tubes of PE-2
and
2 tubes of CMM
Incubate at 30-35°C up to 14 days

Thermophiles

2 tubes of PE-2
and
2 tubes of CMM
Heat shock one tube of each medium at 80°C for 10 minutes
And incubate at 55°C for up to 7 days

For suspected positive and positive tubes

↙ ↙ ↘ ↘

Gram stain

Direct Microscopic growth Examination

Aerobic growth

Duplicate PCA

Incubate for 2 to 5 days

Anaerobic growth

Duplicate LVA or RCM

Incubate for 2 to 5 days

If there is growth (Aerobic or Anaerobic)

↙ ↓ ↘

Gram stain

Identification

Cell morphology for each colony

Diagram 2. Microbiological Analysis of the Contents of Canned Foods that are Acidic or have been Acidified using Aerobic and Anaerobic Media.

Swollen Container
(Use containment devices. Handle as if it contained *C. botulinum*)
or
Non-swollen Container

↓

Cleaning and Disinfection

↓

Liquid Aerobic/Anaerobic Media

↙ ↘

Mesophiles

2 tubes of Acid Broth
and
2 tubes of the sterilized food
Incubate one of each tube type
aerobically and one anaerobically
Incubate at 30-35°C for at least 14 days

Thermophiles

2 tubes of Acid Broth
and
2 tubes of the sterilized food
Incubate one of each tube type
aerobically and one anaerobically
Heat-shock one tube of each medium at
80°C for 10 minutes and then incubate at
55°C for up to 7 days

For suspected positive and positive tubes

↙ ↙ ↘ ↘

Gram stain

Direct Microscopic growth Examination

Aerobic growth

Anaerobic growth

↓

Duplicate plates of PDA and Thermoacidurans agar or SDA and Orange Serum Agar. Incubate 1 plate of each aerobically at 35°C and 55°C and 1 plate of each anaerobically at 35°C and 55°C, for 2-5 days.

If there is growth

↙ ↓ ↘

Gram stain

Identification

Cell morphology for each colony

Diagram 3. Microbiological Analysis of Canned Foods using the Direct Plate Method

Swollen Container
(Use containment devices. Handle as if it contained *C. botulinum*)
or
Non-swollen Container

↓

Cleaning and Disinfection

↓

Direct plating media

↙ ↘

Low acid foods

Duplicate plates of PCA and LVA or RCM if needed, any specific medium e.g. Baird-Parker, MacConkey, etc.

Acid foods

Duplicate plates of PDA and TA or SDA and Orange Serum Agar

Incubate one plate of each medium aerobically and one anaerobically
Incubate at 35°C for 2-5 days

If there is growth

↙ ↓ ↘

Gram stain

Identification

Cell morphology for each colony