

**United States Department of Agriculture**

**Food Safety and Inspection Service**

**MLG 10.01**

**Examination of Canned and Aseptically Processed,  
Hermetically Sealed Meat and Poultry Products**

Laboratory  
procedure for  
performing  
Examination of  
Aseptically  
Processed,  
Hermetically  
Sealed Meat and  
Poultry Products

# MLG 10.01 Examination of Canned and Aseptically Processed Hermetically Sealed Meat and Poultry Products

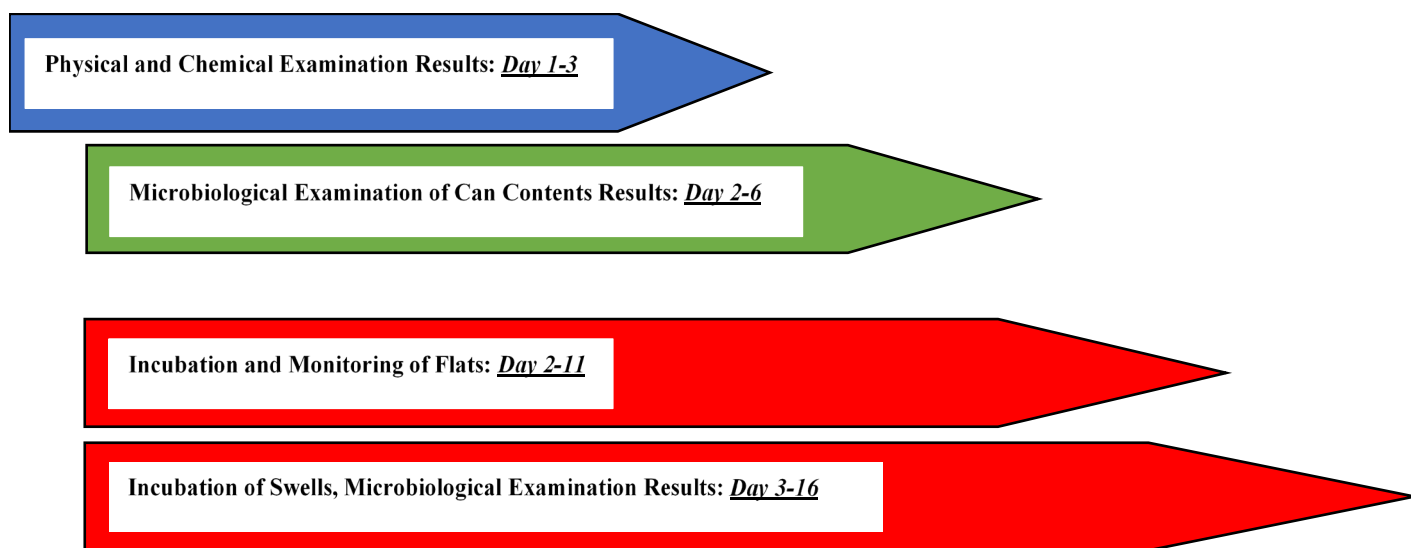
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## Introduction

This method describes how to find and classify container, content, and processing defects, which may lead to spoilage in aseptically processed, hermetically sealed packaging for meat and poultry products. The history of the product, condition of container, odor, appearance of contents, pH, types and number of microorganisms are all used to determine a probable cause of spoilage. Key definitions are included throughout the method for reference. Results for an investigation can take from one day for an obvious physical defect to 16 days to rule out a defect. A timeline for the process is illustrated below. For an extensive flowchart of the timeline see MLG 10 Appendix 1.

### Reporting Timelines:



### KEY DEFINITIONS

**“Canned” foods: meat and poultry** food products that have been preserved by heating in hermetically sealed containers. Those containers are designed to provide a hermetic seal that can protect the product from the entry of microorganisms. These containers can include:

**Traditional Rigid Containers:** for example, metal cans and glass jars

**Semi-Rigid Containers:** for example, plastic or multi-laminate paper cans, bowls and trays

**Flexible Containers:** for example, retortable pouches and bags

**Shelf Stability:** the condition achieved by application of heat, alone or in combination with other ingredients and/or treatments, to render the product free of microorganisms capable of growing in the product at non-refrigerated conditions (over 10°C) at which the product is intended to be held during distribution and storage. “Shelf Stable” is synonymous with “commercially sterile”.

**Hermetically Sealed Container:** an air tight container that is totally sealed to prevent the entry or escape of air and therefore secure the product against the entry of microorganisms during and after thermal processing.

**Cured Meat/Poultry Products:** products containing curing agents which are usually minimally heat processed and rendered shelf stable by the curing agents

**Uncured Meat/Poultry Products:** products given a thorough heat treatment to ensure at least a 12 log reduction of *Clostridium botulinum* spores.

### Safety Precautions

Due to the possible presence of botulinum toxin, CDC Biosafety Level (BSL)-2 guidelines must be followed. The Safety Data Sheets (SDS) must be obtained from the manufacturer for the media, chemicals, reagents, and microorganisms. CDC Biosafety Level 2 guidelines must be followed whenever live cultures are used. The personnel who will handle the material must read the SDS prior to startup. The analyst should wear safety glasses, preferably with some type of face shield, when opening swollen cans.

**Table 1: Equipment and Materials Listed by Method Step**

<b>Step</b>	<b>Materials</b>
<b>Initial Area Preparation</b>	Personal protective equipment, face shield, Class II laminar flow hood or biosafety cabinet
<b>Initial Evaluation of Container</b>	Permanent marker, 5X magnifier, analytical balance with sensitivity to at least $\pm 0.1$ g
<b>pH</b>	pH meter preferably equipped with a flat electrode, sensitivity to at least $\pm 0.1$ pH units
<b>Hydrogen and CO2 Tests</b>	Clean test tube, flame, gas cylinder clamp
<b>Vacuum Leak Test</b>	Microleak detection system for metal containers consisting of a transparent acrylic plate with a hole and tubing to a vacuum source and rubber inner gasket that is cut to individual container sizes and diameters. Bituminous compound in strips (tar type strips usually available in hardware stores) or modeling clay, Wooden dowels, 1/2" diameter (1.27 cm), gas cylinder clamp
<b>Tear-Down and Seam Analysis</b>	Waco video seam monitor, Universal can stripper (WACO Wilkens-Anderson Co Cat, 10700-FC or equivalent), Waco seam saw or equivalent (WACO- Wilkens-Anderson Co), micrometer calipers, countersink meter, metal plate scissors, nippers, sonic cleaning apparatus, commercial dishwasher
<b>Preparation of Containers Prior to Opening</b>	Scouring pad containing a soap solution, abrasive cleaner, sterile towels, tap water, large autoclavable holding pans
<b>Opening Cans Aseptically</b>	Sterile Bacti disc cutter or another suitable opening device (Terriss Consolidated T-14-182), large autoclavable holding pans
<b>Sampling of Container Contents</b>	Sterile dairy trier, cork borer, scissors, scalpels, knives, spoons, 8" forceps, pipettes, specimen containers, aluminum foil, and small wire baskets or trays (to hold pouches in an upright position)
<b>Culturing of Container Contents</b>	Sterile cotton swabs, incubators 20, 35, & $55 \pm 2^\circ\text{C}$ , anaerobic incubation system
<b>Microscopic Staining and Examination</b>	Microscope, microscope slides, immersion oil and cover slips, pipettes

**Table 2: Media and Reagents**

<b>Reagent</b>	<b>Purpose</b>
<b>Seamtest Type U (Liquid Concentrate) or equivalent</b> <i>Winton Products Co., Inc.</i>	Leak detection
<b>Modified Cooked Meat Medium (MCOMM)</b>	Cultivation of aerobic, microaerophilic, and anaerobic microorganisms, especially <i>Clostridium</i> species (Neutral Red pH indicator changes from Red to Orange to Yellow in the presence of microorganisms).
<b>Bromcresol Purple Broth (BCP) or Dextrose Tryptone Broth</b>	Aerobic growth broth with BCP pH indicator which changes from purple to yellow due to acid production. Also used for Cultivation of thermophilic "flat sour" microorganisms related to the spoilage of food.
<b>Plate Count Agar</b>	Enumeration of organisms
<b>All Purpose Medium with Tween® (APT) Agar</b>	Cultivation of heterofermentative lactic acid bacteria including Lactobacilli
<b>Kenner Fecal (KF) Broth</b>	Cultivation of Enterococci
<b>Gram Stain reagents/kit</b>	Classification of gram-positive bacteria and gram-negative bacteria
<b>Endospore Stain</b>	Identification of the presence of endospores
<b>5% Sheep's Blood Agar (SBA)</b>	Cultivation of fastidious organisms
<b>AnaeroPack™ System</b> <i>Thermo Scientific™ R681001</i>	Cultivation of anaerobic organisms
<b>VITEK® 2 ANC ID card</b> <i>www.biomerieux-diagnostics.com</i>	Biochemical identification cards for anaerobes
<b>Dishwashing detergent</b>	Cleaning surfaces
<b>Chlorine Solution</b> <b>0.05% minimum concentration,</b> <b>(Commercial Bleach with approximately</b> <b>5-8% available chlorine diluted 1:100</b> <b>with R.O. Water or equivalent)</b>	Sporicidal disinfection of surfaces
<b>Cryogenic beads</b>	Extended storage of isolates
<b>Limewater</b>	CO <sub>2</sub> detection

**The equipment, materials, and reagents described in this method are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products or vendors and acknowledges that equivalent products may be available for laboratory use.**

**Part 1 Physical and Chemical Evaluation of Canned Product**

**Initial Evaluation of Container Condition**

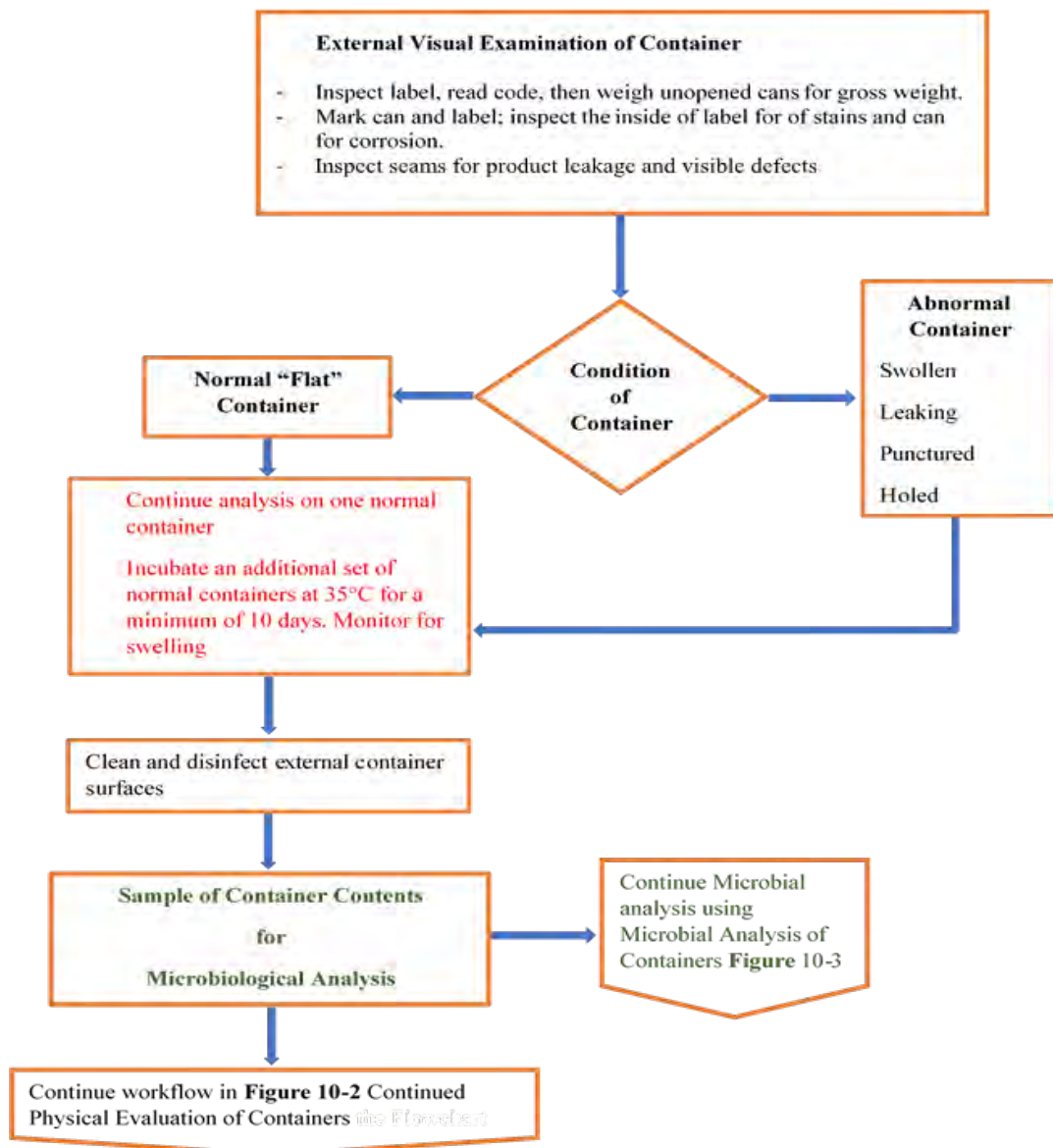
Prior to opening, all containers submitted to the laboratory must be re-evaluated for condition of container. Visually examine the container for any obvious defects. For more detailed information on container defects and abnormalities, see the sidebar “Key Container Resources.” All containers must be at room temperature for classification. Examine normal and abnormal containers from the same production lot for conditions such as “swells” (see key definitions). For containers that exhibit no obvious defects, a tear down and seam analysis may be warranted.

**Key Container Resources**

- [Condition of Containers Examination \(COCE FSIS Dir 9900.2\)](#)
- [The IFSH Package Integrity Internet Information Resource](#)

**Workflow for the Physical and Chemical Evaluation of Containers**

The process for the physical examination of containers is summarized below in **Figure 10-1** Physical Evaluation of Containers.



**Figure 10-1** Physical Evaluation of Containers



## Physical Evaluation/Classification of Metal/Plastic Cans for Defects

### Initial Evaluation of Container

1. Before opening, visually examine the double end seam(s) and side (if present) for structural defects, flaws, and physical damage; record pertinent observations. Look for obvious defects such as pin holes or defective seams.
2. Carefully, run thumb and forefinger around the inside and outside of the double seams for evidence of roughness, unevenness, or sharpness.
3. Using a permanent marker, make three slash marks at irregular intervals across the label and the coded end seam. Remove the label and copy any label code numbers to the side of the container along with a mark indicating the coded end of the can. Correlate any stains on the label with suspicious areas on the side panel of can body by returning the label to its exact position relative to the slash marks.
4. Examine all non-seam areas of the can and ends for any evidence of physical damage. If the code is embossed, carefully examine it for any evidence of puncturing. Circle any suspect and/or defective areas with an indelible pen and record this information.

### Continued Evaluation of Container

After opening the container and starting the microbial testing, determine the pH and perform an organoleptic evaluation. Whenever possible, a "normal" companion container should be examined along with the abnormal one. Under certain conditions that are detailed in each section, perform additional examinations such hydrogen and CO<sub>2</sub> tests, vacuum test, leak test, and seam tear-down and analysis summarized below in **Figure 10-2** Continued Physical Evaluation of Containers.

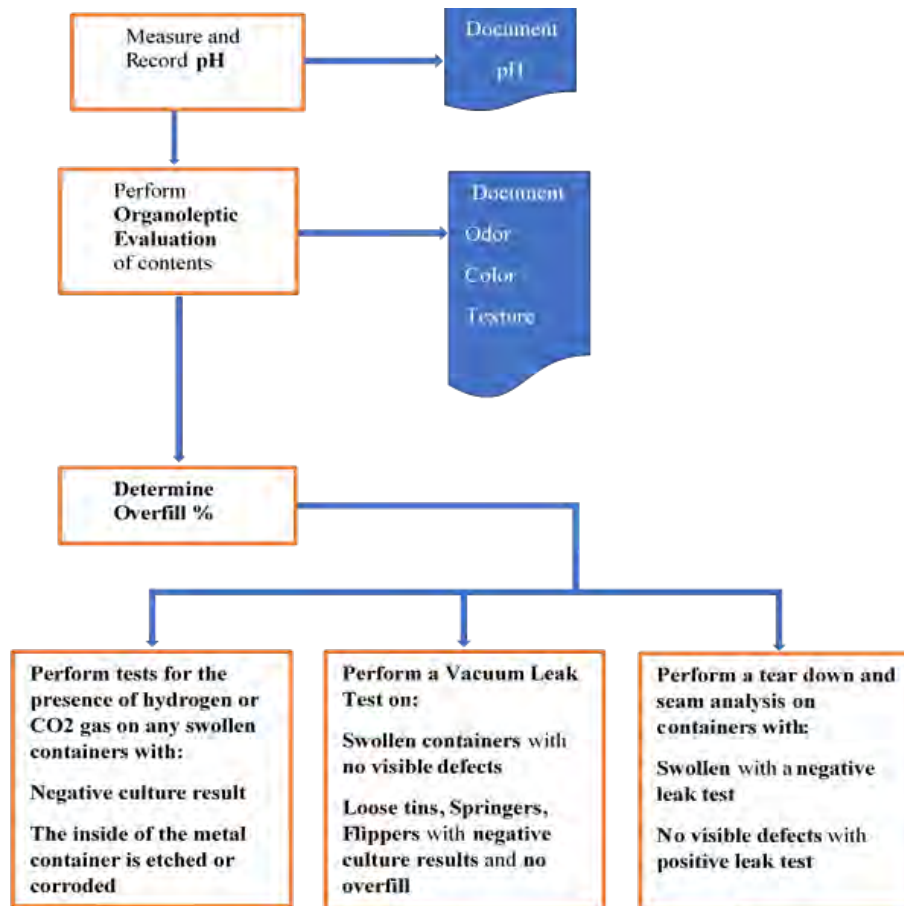


Figure 10-2 Continued Physical Evaluation of Containers



## pH

Determine the pH of the sample, preferably with a flat electrode. After taking this measurement, rinse the electrode thoroughly with deionized or distilled water and disinfect if necessary. For normal pH values of representative products, see MLG 10 appendix 5 Table 1. Document the observed pH.

Document  
pH

## Organoleptic Evaluation

Perform an organoleptic evaluation of the odor and appearance of the sample. Record any abnormalities (See Terms for Documentation of Odor and Appearance below) observed in the container contents, such as off-odors, off-color, changes in consistency and texture when compared with the normal product. At least two analysts should perform an organoleptic examination and compare their evaluations before making a final determination. If the results of odor examination are vague, form an odor panel consisting of at least three trained members. **DO NOT TASTE!**

### Terms for Documentation of Odor and Appearance

**Odor** (e.g., acidic, butyric acid, cheesy, fecal, fermented, medicinal, metallic, musty, pungent, putrid, rotten egg, sharp, sour, sweet)

**Texture** (e.g., tough, hard, soft, digested, slimy)

**Color** (e.g., lighter or darker than the normal control)

**Consistency** (e.g., fluid, viscous, stringy, liquified, coagulated)

**Cooked or uncooked** (compared to normal control)

**Gas production** (e.g. frothy or slight gas production)

**Turbidity** (presence of precipitate in normally clear liquids)

## Determination of Percent Overfill

To determine the percent, overfill:

1. Empty cans thoroughly and run in a dishwasher. Dry can in a 55°C incubator overnight.
2. Weigh empty cans to determine tare weight.
3. Compare tare weight with declared product weight. If the tare weight is greater than the declared product weight, use the following formula to determine the percentage of overfill.

$$\text{Percent overfill} = \frac{(\text{tare weight} - \text{declared product weight}) \times 100}{\text{declared product weight}}$$

4. Document percent overfill of greater than 5% of recorded product weight

Document  
Percent overfill





## Hydrogen and CO<sub>2</sub> Tests

Perform tests for the presence of hydrogen or CO<sub>2</sub> gas on any swollen containers with either or both:

- Negative culture results
- The inside of the metal container is etched or corroded

These conditions could indicate a gas swell.

### Hydrogen Test

1. Hold clean test tube near the can and make a small puncture in the bulging can.
2. Capture some of the escaping gas in the test tube.
3. Invert the tube using your thumb as a seal.
4. Immediately move the mouth of the tube to a lit burner.
5. Vent the gas over an open flame.
6. A popping sound will indicate the presence of hydrogen gas.
7. Document the presence/absence of H<sub>2</sub> gas.

### CO<sub>2</sub> Test

In the same tube as used for the hydrogen test, add a few drops of a saturated aqueous calcium hydroxide solution (limewater). If CO<sub>2</sub> is present, a white precipitate will form on the bottom of the tube. Document the presence/absence of CO<sub>2</sub> gas.

Document  
Presence of H<sub>2</sub> or CO<sub>2</sub>

### Vacuum Leak Test

Perform a vacuum leak test on swollen containers with no visible defects to help identify the location of a leak. Also perform a vacuum leak test on loose tins, springers, or flippers that have negative culture results (Part 2 Microbiological Examination of Container Contents) and no overfill (<5% of net weight listed on the label recorded net weight). Headspace is required to perform this test.

1. When leakage from double seams or side seams is suspected, remove excess metal from the opened end, leaving a 0.5-1 cm flange.
2. Dry thoroughly, preferably overnight, in the 55 ± 2°C incubator.
3. Add water or leak detection liquid (Seamtest Type U Concentrate diluted 1:300 with distilled water) to the can to a depth of 2-4 cm.
4. Place a microleak detector consisting of a transparent acrylic plate with a vacuum gauge and connector for a vacuum source on the open end of the container.
5. Place a gasket (cut pieces of a rubber inner tube will do) between the apparatus and the can.
6. If the fit is not tight (e.g., end seam is bent), fill in the gaps using bituminous compound or modeling clay that has been placed in a 35 ± 2°C incubator until pliable.
7. Large cans without beading or thin metal cans having a wider diameter than height may collapse when vacuum is applied. To prevent this, use 1/2" wooden dowels cut to the appropriate length to support the can sides. Use bituminous compound on the dowel ends to hold them in place. Generally, 4 dowels are enough for a #10 can. Apply the gasket and any bituminous compound to the open can end and fit the leak detector plate in place.
8. Apply the gasket and any bituminous compound to the open can end and fit the leak detector plate in place.
9. Connect the vacuum and apply 10 inches vacuum to the can.

10. Swirl the liquid to dissipate bubbles formed by gases dissolved in the liquid.
11. Examine seams by covering them with the diluted Seamtest. Leaks are identified by a steady stream of bubbles or a steadily increasing bubble size.
12. After carefully examining all seams for leaks, increase the vacuum to 20 inches vacuum and re-examine the seams.
13. Leave the can under vacuum until a leak appears or for a maximum of 2 h and examine at half-hour intervals.
14. Mark the location of leaks on the can's exterior using a marking pen. When reporting, note which seam and the distance from the side seam, or some other appropriate reference point.
15. Retest swollen containers with a negative leak test by placing the container in an ultra-sonic bath, drying it in an incubator, and re-testing for leaks.

**Document  
Any Leaks**

### **Tear-Down and Seam Analysis of Metal and Plastic Containers**

Perform a tear down and seam analysis on containers with the following situations:

- Swollen containers with a negative leak test
  - Containers with a positive leak test with no visible defects
1. After a reserve sample has been taken and all examinations are complete, discard any remaining product into an autoclavable bag and decontaminate by autoclaving.
  2. Use a commercial dishwasher to thoroughly clean the inside of the container. Use an ultra-sonic bath to dislodge any food particles that are in crevices. *Do not autoclave the container since this may destroy any defects.*
  3. After the container is cleaned, dry overnight in an incubator to allow any remaining liquid to evaporate and perform a tare weight.
  4. Examine the interior lining of metal containers for blackening, detinning and pitting.

### **Double Seam Measurements**

For swollen containers with a negative leak test or containers with no visible defects showing a positive leak test, perform a tear-down examination of the double seams.

1. Using a seam analysis tool such as a video seam monitor, measure seam height, seam width, cover hook, body hook, and overlap.
2. Take measurements in thousands of an inch at 2 locations on the uncoded (U) end and 2 positions on the coded (C) end: U7 and U11, and C7 and C11. These positions are determined when looking at the end of container in relation to the side seam (12 o'clock position).
3. The 12 o'clock position is arbitrarily selected for a two-piece container. Specifications for these measurements are determined by the manufacturer for the container diameter, measured in inches (e.g. a 208 diameter container measures 2 and 8/16 of an inch).

**For guidance on the examination of the double seams refer to the following references:**

**Double Seam Inspection and Evaluation Sanitary Cans. 1978. American Can Company, No. 4800-S, or Canned Foods: Principles of Thermal Process Control, Acidification and Container Closure Evaluation**

**Document  
Seam height, width, cover hook**



## Double Seam Teardown for Internal Inspection

1. Perform a visual inspection of the internal seam formation. Look for any seam defects.
2. Check for the presence of sealing compound in metal containers.
3. Visually inspect the flatness of the cover hook. The tightness (wrinkle) is rated from 0-100%.
4. Visually check for drooping at the crossover, which correlates to the % juncture rating.
5. Inspect the pressure ridge. The pressure ridge should be visible and continuous.

Each packer may have different specifications for the finished seams. Contact the in-plant inspector to obtain container specifications.

**Note:** Evaluate double seam overlap and tightness on plastic containers. *Do not remove* the cover hook from the plastic container body to prevent deformation of the hook. In plastic containers, cover hook wrinkles are not an indication of double seam tightness.

### Document

Seam defects such as insufficient cover hook tightness, absence of continuous pressure ridge, excessive drop of the cover hook at the crossover, or body/end fractures

### COMMON SEAM DEFECTS

**INSUFFICIENT COVER HOOK TIGHTNESS**

**ABSENCE OF CONTINUOUS PRESSURE RIDGE**

**JUMPED SEAM**

**EXCESSIVE DROP OF THE COVER HOOK AT THE CROSSOVER**

**BODY OR END FRACTURES**

For a glossary of terms see [MLG10 Appendix 2](#).

## Physical Examination/Classification of Glass Jars

Classify glass jars by the condition of the lid (closure) only. Do not strike a glass jar against a surface. Shake the jar brusquely to cause the contents to exert force against the lid; inspect the contents through the glass prior to opening. Compare the contents of the abnormal/questionable jar with the contents of a normal jar (e.g., color, turbidity, and presence of gas bubbles), and record observations.

1. Before opening, remove the label. Examine the container using a good light source, such as a microscope light, for apparent or suspected defects.
2. Microorganisms may enter jars through small cracks in the glass. Make note of any residue observed on the outer surface and the location. If the button on the center of the lid is popped out, the contents have been compromised.
3. Prior to opening, draw a line with a permanent marker from the metal lid to the side of the glass container. This line serves as a site of orientation for security (tightness) of the container after the jar has been opened.
4. Test the closure gently (e.g. closure is finger-tight) to determine its security. Security is considered positive if the line on the cap is to the right of the line on the container and negative if the line on the

### COMMON GLASS JAR DEFECTS

**CAP TILT:** a press-on/twist-off cap that is not level, showing a cocked or tilted alignment on the glass container.

**COCKED CAP:** found in both lug cap and continuous thread (CT) screw caps. The cap is cross threaded and becomes cocked.

**FLIPPER:** looks normal but when the glass container is shaken abruptly the cap "flips out" (bulges). It returns to its original appearance with mild thumb pressure.

**SWELL:** a glass container with a bulging cap.

**SPRINGER:** a glass container where the cap is convex. Pushing in on the cap, will return it to a concave position. When the outside pressure is released will return to the convex position.

For a glossary of additional terms see [MLG10 Appendix 3 and Condition of Containers Examination \(COCE FSIS Dir 9900.2\)](#).



cap is to the left of the line on the container. Security is measured in 1/16th inch.

5. After sampling has been completed, examine the lid (closure) and the glass rim (sealing surface) of the jar.
6. Examine the gasket impression. Look for flaws in the sealing ring or compound inside the closure, food particles lodged between the glass and the lid, and chips or uneven areas in the glass rim.

#### Document

Security, cracks in the glass, flaws in the sealing ring or compound, chips or uneven areas in the glass rim

### Physical Examination of Pouches

1. Examine the pouches with an illuminated 5X magnifier.
2. Place the pouch on a hard-flat surface (like a table). Firmly place your hand on the surface of the pouch. Examine the pouch for abnormalities (e.g. swelling, leakage, overfilling), and defects (e.g. delamination, severe distortion). Record any observations.
3. Grasp the pouch at both ends and examine both sides for noticeable cuts, cracks, scratches, food residues, punctures, missing labels, foreign materials, or other abnormalities.
4. Examine all seal areas for incomplete fusion. Look for entrapped product, wrinkles, moisture and foreign material. Pay particular attention to the final or closing seal.
5. Circle all actual and suspected defects with a permanent marker to enable additional examination of the defect after all sampling is complete. *A leak test using vacuum is never performed on pouches.*
6. Microscopically examine the pouch for potential leakage sites by looking for points of light coming through the film.

#### Document

Abnormalities: swelling, leakage, overfilling, delamination, severe distortion, noticeable cuts, cracks, scratches, food residues, punctures, missing labels, foreign materials  
 Seal areas: incomplete fusion, entrapped product, wrinkles, moisture and foreign material  
 Microscopic leaks

### COMMON POUCH DEFECTS

**SWOLLEN PACKAGE:** a package that has a distended shape or is ruptured due to internal gas formation.

**NON-BONDING:** a seal showing failure of the two sealant films to combine during the sealing process. The seal impression will be faint or incomplete.

**FRACTURE:** a break in the packaging material.

**WRINKLE:** a fold of material in the seal area.

**SEAL CREEP:** a partial opening of the inner border of the seal. This problem is normally detected by applying some pressure upward toward the seal.

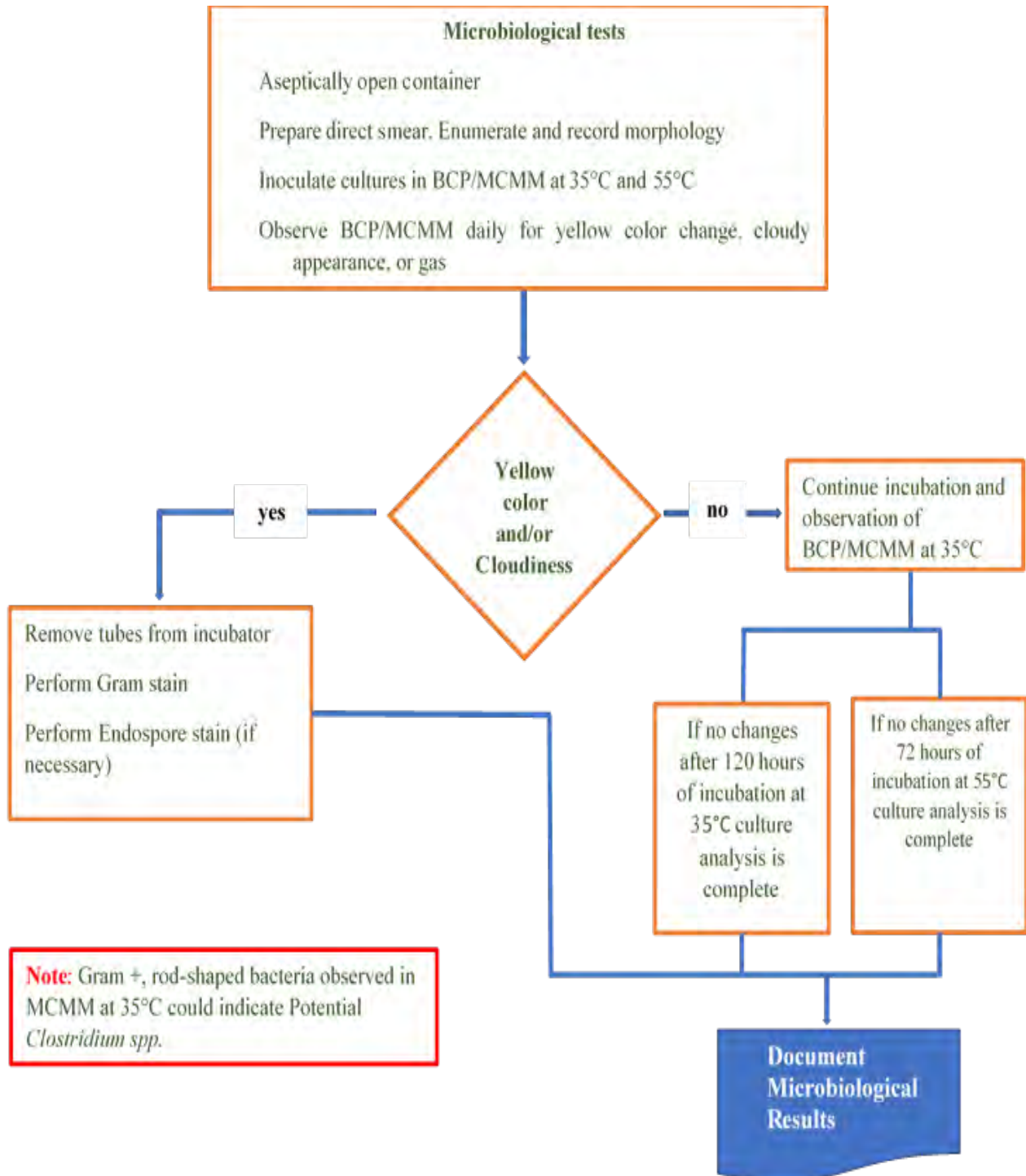
**DELAMINATION:** a separation of the laminate materials forming the package.

For a glossary of additional terms see [MLG10 Appendix 4, Condition of Containers Examination \(COCE FSIS Dir 9900.2\)](#), and [IFSH Package Integrity Resource](#)

**Part 2 Microbiological Examination of Container Contents**

**Introduction**

A thorough microbiological examination of the number and types of organisms in the container contents are critical in an accurate determination if or how microorganisms caused spoilage. The following procedures are to be followed carefully. The process flow for the microbiological analysis of canned products is summarized below in Figure 10-3.



**Figure 10-3** Microbial Analysis of Containers



## QUALITY CONTROL



### Method Controls

For each medium and temperature, a positive culture and an uninoculated media control must be used from the start of analysis. The positive control for Modified Cooked Meat Medium (MCMM) at  $35 \pm 2^\circ\text{C}$  is *Clostridium sporogenes* ATCC 11437 and for MCMM at  $55 \pm 2^\circ\text{C}$  is *Thermoanaerobacterium thermosaccharolyticum* ATCC 7956. The positive control for Bromocresol Purple Broth (BCP) at  $35 \pm 2^\circ\text{C}$  is *Enterococcus faecalis* ATCC 19433 and for BCP at  $55 \pm 2^\circ\text{C}$  is *Geobacillus stearothermophilus* ATCC 7953. Use fresh cultures and directly inoculate into the media at the time of analysis. Culture maintenance by transferring into BCP broth is not recommended since cultures do not retain viability after a few weeks.

Further testing of positive test results for each respective media and temperature require that the positive control be tested along with the test sample. For example, gram stains and biochemical identification require that the method controls are tested from the inoculated media control simultaneously with the test sample.

### Specific Procedure Controls

Each step of the analysis requires the use of appropriate controls to verify that the results are valid. Biochemical kit and rapid test manufacturers may specify control cultures for use with their products. If not specified, quality control procedures for biochemical tests and test media should include cultures that will demonstrate pertinent characteristics of the product.

### Preparation of Containers

#### Preparation of Containers Prior to Opening

1. Record the gross weights of all containers.
2. Separate and record all containers by code numbers, product and container condition.
3. Examine the exterior condition of the container and record any container defects (see the Evaluation of Container Condition page 6).
4. For metal containers that will be opened, remove the wrap-around label, if applicable, and save one for future reference.
5. Open the uncoded end of metal containers. Label the side and top of the coded end with the internal lab number and sub sample letter using a permanent marker pen. For flexible pouches, label the side that is not opened.

#### Preparing Metal Cans

1. Chill swollen metal cans in a refrigerator at  $5 \pm 3^\circ\text{C}$  prior to preparation.
2. Scrub the uncoded end of the metal can with abrasive cleaner or a scouring pad with soap solution to remove bacteria-laden oil and protein residues.
3. Record the code exactly as printed on the can. **Note:** Do not open the coded end of the container.
4. Sanitize the un-coded end by placing sterile towels saturated with chlorine solution over the cleaned end for at least 15 minutes. Alternatively, immerse the cleaned end in an autoclaved shallow pan containing the chlorine solution.



## Preparing Glass Jars with Metal Closures

1. Scrub the surface of the jar closure with a scouring pad containing soap solution.
2. Rinse well with water (tap, DI, or Distilled).
3. Sanitize the jar closure by placing sterile towels saturated with chlorine solution over the cleaned end for at least 15 minutes. Alternatively, immerse the cleaned end in an autoclaved shallow pan containing the chlorine solution.

## Preparing Rigid/Semi-Rigid Plastic Containers

1. Scrub the bottom surface of the container with a scouring pad containing soap solution.
2. Rinse well with tap water.
3. Sanitize the jar closure by placing sterile towels saturated with chlorine solution over the cleaned end for at least 15 minutes. Alternatively, immerse the cleaned end in an autoclaved shallow pan containing the chlorine solution.

## Preparing Normal and Abnormal-Appearing Flexible Retortable Pouches

1. Clean the outside of the pouch with a sterile towel containing a soap solution and rinse well.
2. Sanitize the entire pouch in a suitably sized autoclaved pan by immersion in a chlorine solution for at least 15 minutes. then wipe dry with sterile towels.

## Sampling of Container Contents

The sampling and transfer processes must be conducted aseptically; care must be taken to prevent contamination during the various manipulations. Refer to the Safety Precautions section page 4 prior to beginning the sampling and transfer process.

## Normal-Appearing Metal Cans and Glass Jars with Metal Closures

1. Prepare the container as described in the previous section.
2. Shake the container to distribute the contents.
3. For metal containers, use a sterilized opening device (e.g. Bacti-disc cutter) to cut the desired sampling hole size. For glass jars with metal closures, aseptically unscrew the pre-sterilized lid. Using a pipet or sterile spoon, aseptically transfer a 30-50 ml or 30-50 g portion of the product to a sterile specimen container, or equivalent sterile container for use as a working reserve.
4. Transfer samples immediately to MCMM and BCP media with a sterile pipette or swab and proceed as outlined in Culturing of Container Contents section on page 15.

**Caution:** The contents from overfilled cans may flow out of the hole onto the surrounding lid surface at the time of opening. This material can then drain back into the can when the opening device is removed. Should this occur, terminate the analysis.

## Swollen Cans

1. Cans displaying a hard swell should be chilled in a refrigerator at  $5 \pm 3^{\circ}\text{C}$  before opening. Most foods spoiled by *Geobacillus stearothermophilus* will not produce gas (flat sour spoilage). However, if nitrate or nitrite is present in the meat/poultry product, gas may be produced by this microorganism. Cold temperatures will frequently kill *G. stearothermophilus* resulting in no growth in Bromcresol Purple Broth. Depending on the number of cans sent, save one or two cans and store without refrigeration.



2. ***NEVER FLAME A SWOLLEN CAN - IT MAY BURST.*** Place the can to be opened in a large, shallow, autoclavable pan. The side seam, if present, should be facing away from the analyst. A container with a hard swell may forcefully spray out some of its contents, posing a possible hazard to the analyst if the contents are toxic. Therefore, these cans should be considered a biohazard and precautions must be taken to protect the analyst. Protective gloves should be worn, and the lab coat should be tucked inside the cuffs of the gloves or at least secured around the wrist. Some type of facial shield is also recommended.
3. To prevent violent leakage, place the sanitized can into a biohazard bag and cover with a sterile towel. Invert a sterile funnel with a cotton filter in the stem over the can. Place the point of the sterile opening device in the middle of the can closure. Make a small hole in the center of the sterilized end/closure. Try to maintain pressure over the hole. Release the instrument slowly to allow gas to escape into the towel or funnel.
4. After the gas pressure has been released, enlarge the opening to the desired size to permit sampling and aseptically remove some of the container contents. Aseptically transfer approximately 30-50 ml or 30-50 g portion of the product to a sterile specimen container as a working reserve. Transfer samples immediately to MCMM and BCP media with a sterile pipette or swab and proceed as outlined in the Culturing of Container Contents section on page 15.

### **Rigid/semi-Rigid Plastic Containers**

1. Immediately after removing the container from the chlorine solution, wipe off the excess liquid. Then, using a sterilized opening device, cut the desired size entry hole at a container site not affecting the closure site.
2. Aseptically transfer approximately 30-50 ml or 30-50 g portion of the product to sterile specimen container as a working reserve. Transfer samples immediately to the selected media with a sterile pipette or swab and proceed as outlined in the next section Culturing of Container Contents.

### **Flexible Retortable Pouches**

1. Place the disinfected pouch on a sterile towel with the coded flat side face down. Using sterile scalpels or scissors and forceps, cut a square flap sufficient in size to easily remove product for testing. Do not cut a complete square because a tare weight is required of the empty pouch. The cut should also be at least one quarter of an inch from the seam edge so as not to affect seam inspection for defects. Aseptically transfer approximately 30-50 ml or 30-50 g portion of the product to a sterile specimen container as a working reserve.
2. Transfer the samples immediately to the selected media with a sterile pipet or swab and proceed as in in the next section Culturing of Container Contents.

### **Culturing of Container Contents**

The sampling and transfer processes must be conducted aseptically; care must be taken to prevent contamination during the various manipulations. The sequence of analytical testing is flats first and abnormal (swollen) containers last. This will ensure no cross- contamination from the swollen containers to the flats. If cross-contamination is suspected, re-culture the sterile reserve.

1. Store the appropriately labeled reserve container in a refrigerator at  $5 \pm 3^{\circ}\text{C}$  for the duration of analysis. After completion of analysis, transfer the reserve to the freezer at  $\leq -10^{\circ}\text{C}$  for long term storage
2. Transfer the sample immediately to the selected media, inoculating each tube at the bottom. Whenever possible, use a pipette and pro-pipette to remove 1-2 ml of product for





inoculating each tube of medium. When the nature of the meat/poultry product makes it impossible to use a pipet or spatula, use a sampling swab (holding it by the very end of the shaft) to transfer 1-2 g of the product to each tube. This is accomplished by plunging the swab into the product, then inserting the swab as far as possible into the appropriate tube of medium and breaking off the portion of the shaft that was handled. Use one swab for each tube of medium. When inoculating MCMM, force the broken swab to the bottom of the tube by using the tip of another sterile swab.

3. On a clean microscope slide, make a smear of the product for direct microscopic examination. Allow to air dry. Heat fix by placing on a heating block until hot to the touch or placing overnight in a  $55 \pm 2^\circ\text{C}$  incubator. Do not overheat the slide.
4. Transfer a portion of the container contents to a sterile Petri plate, clean jar, or beaker for pH and organoleptic examination.
5. For each sample, inoculate 2 tubes of MCMM which were steamed (or boiled) for 10 minutes and cooled just before use and 2 tubes of Bromcresol Purple Broth. If a tube of KF medium is inoculated at the same time, the presence of enterococci can be determined rapidly.
6. As a process control, label two uninoculated tubes of each medium to serve as controls. If multiple samples are cultured at the same time, only one set of control tubes are needed for each medium and each temperature.

### Incubation of Culture Media

1. Incubate one tube each of MCMM and BCP for a maximum of 5 days at  $35 \pm 2^\circ\text{C}$  and one tube each for a maximum of 3 days at  $55 \pm 2^\circ\text{C}$ . If used, incubate the tube of KF medium for a maximum of 5 days at  $35 \pm 2^\circ\text{C}$ . For the MCMM and BCP controls, incubate one tube at  $35 \pm 2^\circ\text{C}$  and one at  $55 \pm 2^\circ\text{C}$ . Observe and record growth/no growth from all tubes at 24h intervals for the duration of incubation. Incubation can be stopped once growth is observed.

Typically, only aerobic or facultative organisms will grow in BCP. Generally, only anaerobic or facultative organisms will grow in MCMM. Growth in BCP/KF appears cloudy with acid production (BCP broth changes from purple to yellow). Growth in MCMM appears cloudy with acid/gas production (broth and meat pellets change from red to yellow. Bubbles from the meat indicate gas production).

2. Subculture any questionable tubes, especially if the product under examination exhibits turbidity or if color change is observed in the culture media after observing a clean direct slide. A color change in the media (e.g. BCP changes from purple to yellow) with no cloudiness may be due to pH change from the product and not from microbial growth.

**Document**  
Growth/no growth, color change



## Analysis of Flat Containers

After the analysis of one of the normal cans, all the remaining "normal" cans are incubated at  $35 \pm 2^\circ\text{C}$ . Examine the incubated cans daily for up to 10 days. Remove any swells from the incubator as they develop and culture them along with a normal control. Follow the protocol sequence as described in Sampling of Container of Contents for Swollen cans on page 15. This incubation may reproduce the abnormality and thereby document progressive microbiological changes in the product. After the 10-day incubation period, cool the cans to room temperature and reclassify the container condition. Containers received swollen, buckled and blown should never be incubated, but analyzed as described in Sampling of Container of Contents for Swollen cans on page 15 along with a normal control. Incubation temperatures greater than  $35 \pm 2^\circ\text{C}$  should not be used unless thermophilic spoilage is suspected. If containers were stored under elevated warehouse conditions, it is recommended to incubate at  $55 \pm 2^\circ\text{C}$  as well.

## Identification of Organisms

Use the following conventional bacteriological procedures to characterize the type(s) of microbial flora found in the contents of the container. In the description of slides, use terms such as mixed culture or pure culture, anaerobic (MCOMM) or aerobic (BCP) growth, spore former or non spore former, mesophile or thermophile, cocci or rods. Record all biochemical test results in addition to any characteristic growth patterns on differential and/or selective media. Refer to MLG 10 appendices 4, 5, and 6 for more culture interpretations.

### Direct Smear

From the previously heat-fixed direct smear (step 3), flood the slide for 1 minute with crystal violet or methylene blue stain. For direct smears, a Gram stain is of no value since the age of the cells is not known and Gram-stain reactions may not be dependable in the case of old cells. Under 1000X oil immersion observe at least 5 different fields for the presence of rods, cocci, free spores, or spores within vegetative cells (endospores). For each field, perform a differential count of the morphological types to determine the initial level of bacterial growth in the food itself. If the contents of a swollen container show signs of digestion and few bacterial cells, prepare an endospore stain.

**Document  
Numbers and morphology (rods, cocci, free  
spores or endospores)**

### Endospore Stain

An endospore stain can be used for better definition of spore type and placement. The most common endospore stain is the use of malachite green with moist heat as the mordant and gram safranin as the counter stain.

1. Prepare a new smear and allow to dry.
2. Heat fix by passing the slide through a low flame 2-3 times or placing on a heating block until hot to the touch. Do not overheat the slide.
3. Place slide over boiling water until definite drops of water collect on slide bottom.
4. Flood slide with Solution A (malachite green).
5. After 1 to 2 min, wash slide thoroughly in cool tap water.



6. Counterstain with Solution B (Safranin O) for 20-30 sec, rinse slide thoroughly in cool water.
7. Dry without blotting and examine microscopically under oil immersion at 1000X.
8. Endospores retain the primary stain (malachite green) and vegetative cells the pink counterstain (safranin). Refer to MLG Appendix 1 for preparation of solutions.

**Document  
Presence of Endospores**

## Gram Stain

Perform a gram stain on inoculated media on the day that tubes exhibit growth or production.

1. On a clean glass slide, prepare a smear using a 10 µl loopful from an 18-24 h broth culture. If selecting from an isolated colony, emulsify a colony from an 18-24 h plate in saline to obtain a thin, uniform smear. A touch from an inoculating needle of an isolated colony is generally enough to yield enough cells for viewing microscopically. Allow the smear to air dry. Heat fix by passing the slide through a low flame 2-3 times, placing on a heating block until hot to the touch, or placing overnight in a 55 ± 2°C incubator. NOTE: Do not overheat the slide; excessive heating will cause atypical staining.
2. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 min.
3. Remove the primary stain by gently washing with cold tap water.
4. Flood the slide with mordant (Gram Iodine) and retain on the slide for 1 min (a longer time won't negatively affect the gram stain reaction).
5. Remove the mordant by gently washing with tap water.
6. Decolorize (Gram Decolorizer) until solvent running from the slide is colorless (3-60 sec). The optimum time for most stains is 10-20 sec.
7. Rinse the slide gently in cold tap water.
8. Flood the slide with counterstain (Gram Safranin) and stain for 20-30 sec.
9. Rinse the slide gently in cold tap water.
10. Blot with blotting paper or paper towel or allow to air dry.
11. Examine the smear microscopically at 1000X under oil immersion.
12. Record the morphological types observed and their Gram reaction.

Gram-positive organisms retain the primary stain (crystal violet) and appear blue or purple. Gram-negative organisms retain the counterstain (safranin) and appear red. If endospores are present, the Gram stain can generally detect spores microscopically under 1000X oil immersion. Most spores will not retain any stain and appear as empty vacuoles.

**Document  
Gram Stain results**

## Gas Forming Anaerobes (GFAs)

MCCM tubes showing a bright yellow color with visible gas bubbles and containing gram positive or gram variable rods with/without spores should be suspected of containing GFAs. Spores become

**Document  
Presence of GFAs**



more visible with prolonged incubation. Therefore, if spores are not seen microscopically on initial examination, re-incubate the original tube and check for the presence of swollen endospores.

### Suspect Clostridium Species

Gram-positive, rod-shaped bacteria observed at 35°C in MCMM could indicate *Clostridium* species. For further testing of suspect *Clostridium* species, transfer a positive MCMM tube to SBA and incubate at 35± 2°C for 24-48 h under anaerobic conditions. Perform VITEK® 2 biochemical identification on isolated colonies on SBA. Transfer suspect *Clostridium botulinum* sub-cultures to MCMM and incubate at 35 ± 3°C for 4-5 days. These tubes are forwarded to a predetermined laboratory which has the capability to test for botulinum toxin. The presence of *Clostridium botulinum* in food products shall be reported to the Federal Select Agent Program managed by the Center for Disease Control and Protection (CDC) using [APHIS/CDC Form 4](#).

### Culturing of Canned, Perishable Meat/Poultry Products

Perishable meat and poultry products, such as hams, luncheon meats, and loaves are packaged in hermetically sealed containers and then heat processed to internal temperatures of between 65 and 71°C. These products are not shelf stable. They may contain vegetative cells and/or spores and must be refrigerated. "**Perishable, Keep Refrigerated**" must appear on the label of these products. The process flow for the microbiological analysis of canned perishable meat/poultry products is summarized below in Figure 10-4.

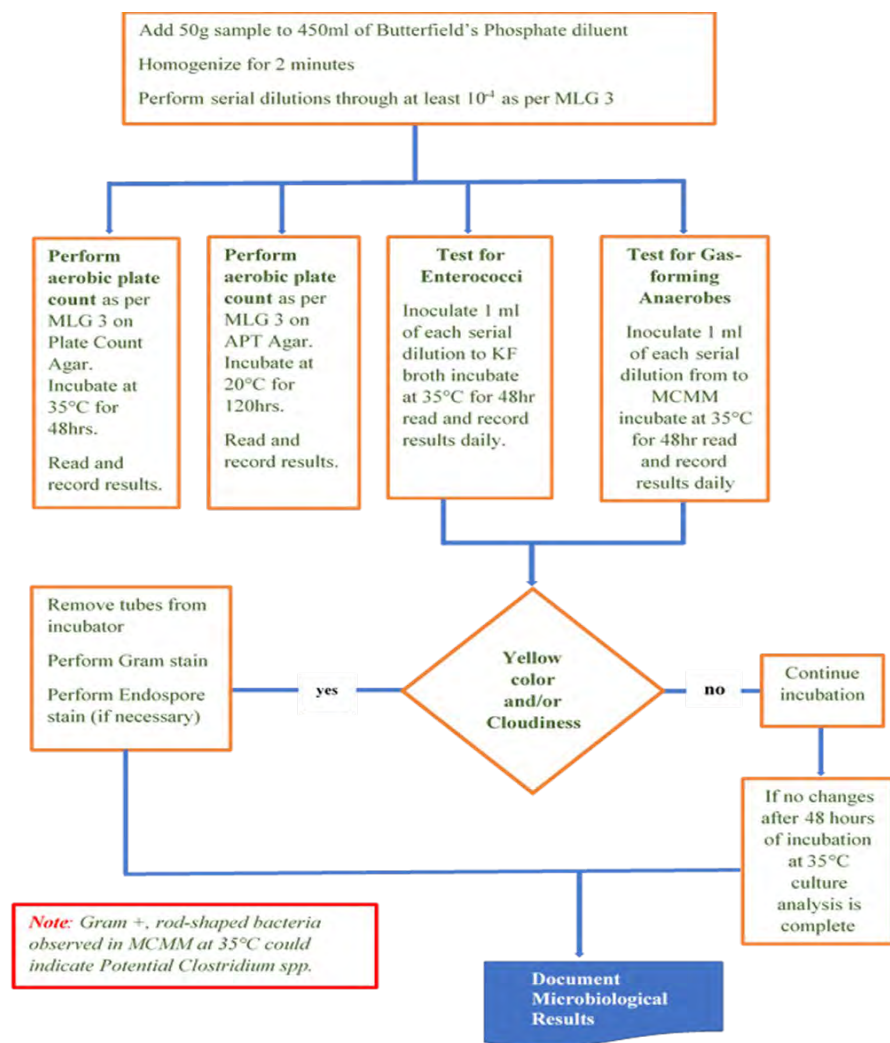


Figure 10-4 Microbial Analysis of Canned Perishable Products



## Sampling of Canned, Perishable Meat/Poultry Products

### Initial Set-up

1. Prepare the container as described in the Preparation of Containers section on page 12. Open the container as described in the Sampling of Container Contents section and remove a  $50 \pm 1$  g of sample with sterilized dairy trier, large cork borers, scissors, knife or forceps.
2. Label a 1.5 ml tube for each sample.
3. Place the sample into a sterile blender jar or stomacher bag, add 450 ml of sterile Butterfield's Phosphate Diluent and homogenize for 2 minutes. This is a 1:10 dilution; make additional dilutions through at least  $10^{-4}$ . Use these dilutions to prepare the Aerobic Plate Count, Gas Forming Anaerobe, and Enterococci
4. After sampling, cover the container opening with sterile aluminum foil several layers thick and secure with tape. Place the opened sample unit in the freezer at  $\leq -10 \pm 2^{\circ}\text{C}$  until the analysis is complete.

### Aerobic Plate Counts

1. Pipet 1 ml of each dilution prepared above in step 3 of the Initial Set-up into each of two sets of duplicate pour plates according to the instructions given in MLG chapter 3.
2. Prepare the first set of plates with Plate Count Agar. Incubate this set at  $35 \pm 1^{\circ}\text{C}$  for  $48 \pm 2$  h.
3. Prepare the second set of plates with APT agar Incubate this set at  $20 \pm 1^{\circ}\text{C}$  for 4-5 days.
4. Count and record the results from both sets as described in MLG chapter 3.

Document  
Plate count results

### Gas Forming Anaerobes (GFAs)

1. Steam tubes of MCMM for 10 minutes and cool just prior to use.
2. Inoculate each tube with 1 ml of each dilution prepared above in step 3 of the Initial Set-up. Begin with the 1:10 dilution and continue with subsequent dilutions. Use a separate pipet for each dilution. Dilutions must be sufficiently high to yield a negative endpoint. Be sure that the inoculum is deposited near the bottom of the tube.
3. Incubate MCMM tubes at  $35 \pm 2^{\circ}\text{C}$  for  $48 \pm 1$ h, read daily.
4. Consider any MCMM tubes showing a bright yellow color, containing visible gas bubbles and containing gram positive or gram variable rods as positive for GFAs.
5. Determine the gas forming anaerobe count using an MPN table (e.g. MLG 3 Appendix 2). Based upon the highest dilution showing these organisms, report the approximate number of gas forming anaerobes per gram, calculated as the reciprocal of the highest positive dilution. If skips occur, disregard the final actual dilution and calculate the end point at the dilution where the skip occurred. This is only an approximation of the gas forming anaerobe count.

Document  
GFA count

If *Clostridium botulinum* is suspected, representative tubes that have not been opened should be reincubated at  $35 \pm 2^\circ\text{C}$  for a total of 4-5 days  $\pm$  1h for toxin production. These tubes are forwarded to a predetermined laboratory which has the capability to test for botulinum toxin. The presence of *Clostridium botulinum* in food products shall be reported to the Federal Select Agent Program managed by the Center for Disease Control and Protection (CDC) using [APHIS/CDC Form 4](#).

## Enterococci

1. Transfer 1 ml of each dilution prepared above in step 3 of the Initial Set-up to individual tubes containing 5-8 ml of KF broth. Use a separate pipette for each dilution. Begin with the 1:10 dilution and continue with each subsequent dilution. Dilutions must be sufficiently high to yield a negative end point.
2. Incubate the KF broth at  $35 \pm 2^\circ\text{C}$  for 48 h  $\pm$  1h. Presumptive positive tubes exhibit a yellow color, and signs of growth e.g. turbidity, flocculence, and/or sediment.
3. Confirm all presumptive positives microscopically. Wet mounts examined under low light or gram stained preparations are suitable for these microscopic determinations. Microscopic determinations yielding cells with ovoid streptococcal morphology shall be considered confirmed positive.
4. Report the approximate number of enterococci per gram, calculated as the reciprocal of the highest positive confirmed dilution. If skips occur, disregard the final actual dilution and calculate the end point at the dilution where the skip occurred. This is only an approximation of the number of enterococci. A minimum of three tubes per dilution and an MPN table (MLG Appendix 2) must be used for a more accurate determination of organisms.

Document  
Enterococci count

## Interpretation of Results

The investigation for final determination of abnormal containers is a collaborative effort involving the producer, field inspectors, policy development staff, and laboratory analysis. The timely and accurate reporting of laboratory results is paramount for determination of final lot disposition. All documented observations and results such as condition of container, odor, appearance of contents, presence of gas, pH, types and number of microorganisms are used to determine a probable cause of an abnormal container. Based on these observations and results, use the following appendices to determine possible causes of spoilage. For low acid (pH range 5.0 to 8.0) foods use **MLG 10 Appendix 6** “Key to Probable Cause of Spoilage in Low Acid Canned Foods”. For semi-acid (pH range 4.6 to 5.0) canned foods use **MLG 10 Appendix 7** “Key to Probable Cause of Spoilage in Semi-Acid Canned Foods”. For perishable canned Meat/Poultry Products use **MLG 10 Appendix 8** “Characteristics of Normal and Abnormal Perishable Canned Meat/Poultry”. Interpretation of results is summarized in the table below.



<b>Determination of Probable Cause of Spoilage</b>		
<b>Documented Information</b>		
<ul style="list-style-type: none"> <li>• Container Integrity Evaluation</li> <li>• pH</li> <li>• Organoleptic Evaluation</li> <li>• Determination of Percent Overfill</li> <li>• Presence of H<sub>2</sub> or CO<sub>2</sub></li> <li>• Vacuum Test Results</li> <li>• Tear Down and Seam Analysis Results</li> <li>• Microbiological Cultural and Enumeration Results</li> </ul>		
<b>Appendix</b>		
<b>Low Acid</b> pH range 5.0 to 8.0	<b>Semi-Acid</b> pH range 4.6 to 5.0	<b>Perishable Canned Meat/Poultry Products</b>
<b>MLG 10 Appendix 6</b> Key to Probable Cause of Spoilage in Low Acid Canned Foods	<b>MLG 10 Appendix 7</b> Key to Probable Cause of Spoilage in Semi-Acid Canned Foods	<b>MLG 10 Appendix 8</b> Characteristics of Normal and Abnormal Perishable Canned Meat/Poultry
<b>Caution:</b> These tables are based on a single cause of spoilage. If there are multiple causes, the tables may not help to determine all the probable causes of spoilage.		

Some common causes for a swollen container are summarized below:

- The most common microbial cause for swollen containers is a lack of hermetic seal due to container defects. This often results in mixed flora growth in MCMM and BCP at 35 ± 2°C, a positive leak test, and abnormal pH and organoleptic results.
- Microbial growth due to improper storage at elevated warehouse temperatures may cause swollen containers. In this case, resulting in culture positive results at 55 ± 2°C.
- Under rare circumstances, buckled cans may be caused by improper cooling temperature or pressure after retorting. In this case, the culture results would be negative, indicating the product is commercially sterile but not suitable for retail marketing.
- Acidic cans may sometimes swell as a result of chemical action. The acids in the food begin to attack and dissolve the metal lining of the can producing hydrogen gas. In this case, the culture results could be negative, and the inside of the metal container may be etched or corroded.

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[Condition of Containers Examination \(COCE\)](#)

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