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MICROBIOLOGICAL TEST METHODS

PART 10 — COMMERCIAL STERILITY OF LOW ACID AND ACID CANNED FOODS

BUREAU OF CEYLON STANDARDS

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SLS 516:Part 10:1983

Gr. 6

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This standard does not purport to include all the necessary provisions of a contract.

SRI LANKA STANDARD

MICROBIOLOGICAL TEST METHODS
PART 10 : COMMERCIAL STERILITY OF LOW ACID AND ACID CANNED FOODS

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Bureau of Ceylon Standards on 1983-04-08, after the Draft, finalized by the Drafting Committee on Microbiological Test Methods had been approved by the Agricultural and Food Products Divisional Committee.

This standard is one of a series of Sri Lanka Standards for Microbiological Test Methods.

This Sri Lanka Standard is intended for general application in the field of the microbiology of foods and feeding stuffs, to products intended for the human consumption or feeding of animals. However, owing to the number of variety of these products, it may be necessary in special cases to make certain changes to these guidelines or even to use other methods. Such changes or other methods will be indicated in the Sri Lanka Standards specific to these products.

All values given in this standard are in SI units.

The assistance gained from the publications of Food and Drugs Administration, USA and Association of Official Analytical Chemists (AOAC), Washington DC in the preparation of this standard is gratefully acknowledged.

1 SCOPE

This Sri Lanka Standard gives a general method for the determination of commercial sterility of low acid and acid foods, packed in hermetically sealed containers such as cans, jars or bottles or flexible pouches of suitable packing material.

2 REFERENCES

SLS 393 Code of practice for handling food samples for microbiological analysis.

3 DEFINITIONS

For the purpose of this Sri Lanka Standard, the following definitions shall apply:

3.1 canned foods : The foods that have been preserved by heat in hermetically sealed containers.

3.2 low-acid food : The foods with finished equilibrium pH value greater than 4.6.

3.3 acid food : The foods with finished equilibrium pH value less than 4.6.

3.4 commercial sterility : The condition achieved by application of heat which renders food free of

a) micro organisms capable of growing in and spoiling the food,

b) pathogenic micro organisms capable of proliferating in the food under normal conditions of storage and distribution.

4 SAMPLING

Sampling shall be carried out in conformity with the relevant Sri Lanka Standard for the product concerned and **SLS 393.**

5 APPARATUS AND GLASSWARE

5.1 Instruments for preparation of samples, sterilized prior to use by autoclaving or in an oven.

5.2 Balance with weights, capacity 2 500 g, sensitivity 0.1 g.

5.3 Apparatus for dry sterilization (oven) or wet sterilization (autoclave).

5.3.1 Other than the equipment which is supplied sterile, particularly what made of plastic material, glassware shall be sterilized either;

- in an oven at 170 ^{o}C to 175 ^{o}C for not less than 1 hour; or
- in an autoclave at 121 ± 1 ^{o}C for not less than 20 min.

5.4 Water bath controlled at 45 ± 0.5 ^{o}C.

5.5 Incubators controlled at 30 ^{o}C to 35 ^{o}C and 25 ^{o}C to 30 ^{o}C.

5.6 Petri dishes made of glass or plastics, diameter 90 mm or 100 mm.

5.7 Total delivery pipettes, having a nominal capacity of 1 ml and 10 ml graduated in 0.1 ml and 1 ml respectively.

5.8 Straight wall pipettes 200 mm to 250 mm long x 7 mm inner diameter 9 mm outer diameter, cut and fire polished or equivalent.

5.9 Disposable or sterile caps (operating room type).

5.10 Can opener (Bacti-disc cutter or bacteriological can opener).

5.11 Cork borer, and

5.12 Usual laboratory equipment.

6 CULTURE MEDIA AND DILUTION FLUID

6.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that for the preparation of culture media, dehydrated basic components or complete dehydrated media should be used. The manufacturer's instructions shall be rigorously followed.

The chemicals used shall be of analytical quality.

Prepare the media with distilled water or if necessary, deionized water provided that it is free of substances which would be toxic of inhibitory under the test conditions.

If the media are not used immediately, they shall be kept in the dark, at a temperature between 0 °C and + 5 °C, for not longer than one month, to avoid any change in their concentration and composition.

6.2 Media for low acid products

6.2.1 *Tryptone broth*

6.2.1.1 Composition (aerobic medium)

Tryptone or trypticase	10.0 g
Glucose	5.0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	1.25 g
Yeast extract	1.0 g
2 per cent alcoholic solution of bromocresol purple	2.0 g
Water	1 000 ml

6.2.1.2 Preparation

Dissolve the components in 1 000 ml water with gentle heat. Dispense 10 ml portions into 20 mm x 150 mm screw-cap test tubes.

Autoclave for 20 min at 121 °C.

6.2.2 *Cooked meat medium*

6.2.2.1 Composition

Bullock's heart	500 g
0.05 N Sodium hydroxide (NaOH)	500 ml

6.2.2.2 Preparation

Minse 500 g of fresh fat-free bullock's heart and add to 500 ml of 0.05 N sodium hydroxide. Mix well and heat to boiling. Simmer for 20 min with frequent stirring. Adjust pH to 7.4. Strain through gauze or muslin, squeeze out excess liquid and dry the meat particles at a temperature below 50 °C. Place dried meat in a 30-ml screw capped container (Universal/McCartney) to a depth of about 3 cm. Add 10 ml of peptone water or nutrient broth. Sterilize by autoclaving at 121 °C for 20 minutes.

6.2.3 *Liver broth*

6.2.3.1 Composition

Fresh beef liver	500 g
Tryptone	10 g
Soluble starch	1.0 g
Dipotassium hydrogen phosphate	1.0 g
Water	1 000 ml

6.2.3.2 Preparation

Remove the fat from the fresh beef liver, grind, mix with 1 000 ml of water and boil slowly for 1 hour. Adjust the pH to 7.6 and remove the liver particles by straining through cheese cloth. Make the volume of the broth back to 1 000 ml with water and add the tryptone, dipotassium hydrogen phosphate and soluble starch. Mix well and refilter. Dispense 15 ml of the broth into 20-mm x 150-mm tubes and liver particles previously removed to a depth of 2.5 cm in each tube. Autoclave at 121 °C for 20 minutes.

6.2.4 *Nutrient agar*

6.2.4.1 Composition

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Water	1 000 ml

6.2.4.2 Preparation

Dissolve the components in 1 000 ml of water and heat to boiling. Autoclave for 30 min at 121 °C.

6.3 Media for acid products

6.3.1 *MRS-broth*

6.3.1.1 Composition

Proteose peptone No. 3	10.0 g
Beef extract	10.0 g
Yeast extract	5.0 g
Glucose	20.0 g
Tween 80	1.0 g
Dipotassium hydrogen phosphate	2.0 g
Sodium acetate trihydrate	5.0 g
Triammonium citrate	2.0 g
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2 g
Manganese sulfate ($MnSO_4.4H_2O$)	0.05 g
Water	1 000 ml

6.3.1.2 Preparation

Dissolve ingredients in water. Adjust medium pH to 6.2 - 6.6 before sterilization for 15 minutes at 121 °C. The pH after sterilization should be between 6.0 and 6.5 MRS agar is prepared by adding 15 g agar per 1 000 ml of medium.

6.3.2 *Sabourauds dextrose broth (SD)*

6.3.2.1 Composition

Polypeptone	10.0 g
Dextrose	40.0 g
Water	1 000 ml

6.3.2.2 Preparation

Dissolve ingredients in water completely and dispense 40-ml portions in prescription bottles. Final pH should be 5.8. Autoclave at 121 °C for 15 min. Do not exceed 121 °C nor autoclave more than 15 min.

6.3.3 *Potato dextrose agar (PDA)*

6.3.3.1 Composition

Potatoes, peeled and diced	200 g
Dextrose (D-glucose)	20 g
Agar	15 g
Water	1 000 ml

6.3.3.2 Preparation

Boil 200 g of peeled, diced potatoes for 1 h in 1 000 ml of water. Filter and make up the filtrate to 1 000 ml. Add the glucose and agar and dissolve by steaming. Distribute (10-ml or 100-ml amounts) in screw capped bottles and sterilize by autoclaving at 120 °C for 20 min.

6.3.4 *Sabouraud dextrose agar*

6.3.4.1 Composition

Dextrose	40.0 g
Peptone	10.0 g
Agar	15.0 g
Water	1 000 ml

6.3.4.2 Preparation

Dissolve ingredients in water and heat to boiling. Dispense in flasks, sterilize in autoclave at 121 °C for 15 min. Final pH should be 5.6 ± 0.2. Do not over autoclave.

7 PROCEDURE

7.1 Incubation

7.1.1 Incubate at 30 °C to 35 °C for not less than 10 days for low acid foods.

7.1.2 Incubate at 25 °C to 30 °C for not less than 10 days for acid foods.

7.2 Preparation of test sample

7.2.1 Conduct tests in a clean room (if necessary open room may be used but outside windows must be closed and direct draughts across work area must be eliminated). If available use laminar flow cabinet.

7.2.2 Remove labels from containers and record details on the label, codes and any evidence of physical damage, particularly to the side and double seams in the case of cans and lids in the case of jars and bottles.

If any of the selected containers show obvious defects due to poor handling during transport, storage and distribution, discard them and replace with containers from the lot.

7.2.3 In case of cans, proceed as follows:

7.2.3.1 Wash cans with soap (or detergent sanitizer solution) and water and dry with clean paper towels.

7.2.3.2 Wipe table top with 100 ppm chlorine solution (eg. chlorox or disodium hypochlorite solution) immediately before placing washed and dried can,on it.

7.2.3.3 Place code end of the can in down position and number cans in ink or with copper sulphate marking solution to right of side seam.

7.2.3.4 Completely cover hair with clean cap. Wash hands with soap and water or detergent sanitizer solution.

7.2.3.5 Flood the non coded end with 70 per cent alcohol pour off excess, play the flame of a bunsen burner down to the end of the can continuing until the visible moisture film evaporates (if the can swells, keep the side seam directing away from the analyst and flame cautiously). Cover the can immediately with a sterile petri dish lid.

7.2.3.6 Clean can opener with 70 per cent alcohol and flame the metal portion. Aseptically cut and remove a disc from the non coded end.

7.2.4 In case of other containers, open them under aseptic condition taking adequate care to prevent contamination.

7.2.5 Immediately remove 2 g or 2 ml of food using a sterile cork borer **(5.11)** for solids or sterile pipette **(5.8)** for liquids and transfer to each of two tubes of aerobic and two tubes of anaerobic media.

NOTE - For acid products inoculate only aerobic media.

7.2.6 Remove additional 25 g or 30 ml of food aseptically, using a sterile spatula or pipette and place in sterile closed containers. Refrigerate at 4 °C for later testing if necessary.

7.2.7 Incubate tubes at 30 °C to 35 °C for 72 h (low acid products) and at 25 °C to 30 °C for 5 days (acid products) and examine.

7.2.8 Subculture tubes showing growth on to plating media and examine.

NOTE - Subculturing is rarely done on a routine basis because it is time consuming expensive and runs a risk of laboratory contamination which could lead to faulty interpretation. This procedure is necessary only when the results obtained in **7.4** *and* **7.5** *are inconclusive.*

7.3 Contamination control

Expose a plate of nutrient agar **(6.2.3)** on table for a time equal to the longest duration that any medium (tube or plate) is exposed. Incubate plates at 30 °C to 35 °C and examine daily for 3 days.

7.4 Naked eye and microscopic examination

7.4.1 Examine and record the odour and naked eye appearance of the contents.

7.4.2 Examine microscopically under oil immersion heat fixed smears of food, stained 10 sec. with 1 per cent gentian (or crystal) violet and washed in running water or alternatively, examine wet mounts with phase contrast microscope. If food contains appreciable fat, xylol should be dripped across the food smear while it is still hot from heat fixing. Compare stained smear with one made from normal products if available.

7.5 pH determination

Determine the pH of the food sample with the pH meter, using the normal pH of the food under investigation as the reference. Record both reference pH and the sample pH. Compare with a normal can of food if available.

7.6 Confirmation

When microbial growth is recorded from a container of food that displays doubtful or no evidence of spoilage, carry out the following confirmation procedure.

7.6.1 Grow the bacterial isolate or isolates on pure culture.

7.6.2 Select an unopened container of food exhibiting the same manufacturers code as the one previously tested.

7.6.3 Using aseptic techniques, make a small puncture hole through the container. (The can ends jar/bottle closure).

7.6.4 Inoculate the product under its surface with the microbial isolate.

7.6.5 Seal the aperture of the container aseptically. In case of cans, flame the puncture hole to create a vacuum in the head space, and aseptically seal with solder on similar material.

7.6.6 Incubate the inoculated container at 30 °C to 35 °C (low acid products) or 25 °C to 30 °C (acid products) for 10 days.

7.6.7 Open the container (7.6.6) and examine the product.

7.7 Interpretation of results

7.7.1 *Commercial non sterility*

7.7.1.1 When direct smears reveal excessive micro organisms and one or more product characteristics (7.4 and 7.5) are abnormal, the product is commercially non sterile.

7.7.1.2 When the results of (7.4 and 7.5) are inconclusive, check on the results of subculture (7.2.7). The product is considered commercially non sterile;

a) when both the tubes in the duplicate set showed growth with similar microbial flora and which in turn is similar to that in the original product; and

b) when the confirmation procedure (7.6) showed that the spoilage indices were indentical for both containers (7.6.2).

7.7.2 *Commercial sterility*

7.7.2.1 When product appears normal to naked eye and microscopy does not reveal excessive micro organisms the product is considered commercially sterile.

7.7.2.2 When the results of (7.4 and 7.5) are inconclusive check in the results of subcultures (7.2.7). The product is considered commercially sterile, under the following conditions:

a) when all the subcultures show no growth;

b) when only one of the tubes in the duplicate set showed growth, the microbial flora of which is not similar to that seen in the original product;

c) when both the tube in the duplicate set showed growth, the microbial flora of which are not similar to each other and/or not similar to that in the original product and the confirmation procedure (7.6) showed an obvious, spoilt product.

NOTE - Viable micro organisms normally may be recovered from commercially sterile heat processed food under three general conditions.

1) The micro organisms is an obligate thermophilic spore forming bacterium and the normal storage temperature is below the thermophile range.

2) The heat processed food is within the high acid to acid range. Acid in tolerant micro organisms may be present but are incapable of growth because of the acidic conditions.

3) Mesophilic or thermophilic spore formers may be recovered from canned foods which are treated by a combined process of heat and water activity control to prevent microbial growth and spoilage of food.

Finding micro organisms in these cases is normal and the product is considered commercially sterile.

8 TEST REPORT

The test report shall show the method used and the results obtained. It shall also mention all operative conditions not specified in this standard or regarded as optional as well as any circumstances that may have influenced the result.

SLS CERTIFICATION MARK

The Sri Lanka Standards Institution is the owner of the registered certification mark shown below. Beneath the mark, the number of the Sri Lanka Standard relevant to the product is indicated. This mark may be used only by those who have obtained permits under the SLS certification marks scheme. The presence of this mark on or in relation to a product conveys the assurance that they have been produced to comply with the requirements of the relevant Sri Lanka Standard under a well designed system of quality control inspection and testing operated by the manufacturer and supervised by the SLSI *which includes surveillance inspection of the factory, testing of both factory and market samples.*

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