SRI LANKA STANDARD 516:PART 4:1982
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MICROBIOLOGICAL TEST METHODS

PART 4 – GENERAL GUIDANCE FOR THE DETECTION AND ENUMERATION OF FAECAL STREPTOCOCCI

BUREAU OF CEYLON STANDARDS

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SLS 516:Part 4:1982

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 4 : GENERAL GUIDANCE FOR THE DETECTION AND ENUMERATION OF *FAECAL STREPTOCOCCI*

FOREWORD

This Sri Lanka Standard was authorized for adoption and publication by the Council of the Bureau of Ceylon Standards on 1982-07-12 after the draft finalized by the Drafting Committee on Microbiological Test Methods has 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 human consumption or feeding of animals. However, owing to the number and 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.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with CS 102.

The assistance gained from publications of International Organization for Standardization (ISO) and the Indian Standards Institution (ISI) in the preparation of this standard is gratefully acknowledged.

1 SCOPE

This Sri Lanka Standard gives general guidelines on two methods for the detection and enumeration of Faecal streptococci in products intended for human consumption or feeding of animals.

a) Plate count method

b) Most probable Number method (MPN).

2 REFERENCES

CS 102 Presentation of numerical values

SLS 393 Handling food samples for microbiological analysis

SLS 516 Microbiological test methods

Part 1 : Aerobic plate count at 36 ± 1 °C

Part 3 : Detection and enumeration of coliforms, and *Escherichia coli*

3 DEFINITIONS

For the purpose of this Sri Lanka Standard, the following definition shall apply.

faecal streptococci : Micro-organisms which form typical colonies on solid selective media and which display the biochemical characteristics described when the tests are carried out according to this method.

4 SAMPLING

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

5 Apparatus and glassware

Usual laboratory equipment and in particular the following shall be used.

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

5.2 *Balance with weights*, capacity 2500 g, sensitivity 0.1 g.

5.3 *Apparatus for homogenization*

5.3.1 *Mechanical blender*, operating at not less than 8000 r.p.m. and not more than 45,000 r.p.m. with glass or metal jars fitted with lids and resistant to the conditions of sterilization. Alternatively a *stomacher* laboratory blender with the sterile bags may be used.

5.4 *Apparatus for dry sterilization (oven) or wet sterilization* (autoclave).

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

- in an oven at 170 °C to 175 °C for not less than 1 hour, or
- in an autoclave at 121 ± 1 °C for not less than 20 min.

5.5 *Drying oven or incubator,* adjustable to 50 ± 1 °C.

5.6 *Water baths,* controlled at 45 ± 0.5 °C and 45 ± 0.1 °C.

5.7 *Incubator,* controlled at 36 ± 1 °C.

5.8 *Colony counter,* dark field model, Quebec or equivalent with suitable light source and grid plate.

5.9 *Tally register.*

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

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

6 CULTURE MEDIA AND REAGENTS

6.1 Basic materials

For uniformity of results, it is recommended that dehydrated basic components or complete dehydrated media should be used for the preparation of culture media. The manufacturer's instructions shall be rigorously followed. Chemicals should be of analytical reagent grade.

Prepare the media with distilled water or if necessary, deionized water provided that it is free from substances which would be toxic or inhibiting under 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, avoiding any change in their concentration and composition.

6.2 Peptone water diluent

Same as in Clause 6.2 of SLS 516 Part 1

6.3 Kanamycin-Aesculin-Azide Agar

6.3.1 *Composition*

Tryptone	20 g
Yeast extract	5 g
Kanamycin sulphate	20 mg
Sodium chloride	5 g
Sodium citrate	1 g
Aesculin	1 g
Ferric ammonium citrate	0.5 g
Sodium azide	0.15 g
Agar	15 g
Water	1 000 ml

6.3.2 *Preparation*

Add ingredients to 1 000 ml of water. Adjust the pH to 7.1 ± 0.1 and heat to boiling to obtain complete solution. Sterilize for 15 min at 121 °C. Cool to approximately 47 °C and dispense 15 ml quantities into sterile petri dishes.

6.4 Tryptose broth

6.4.1 *Composition*

Tryptose	10 g
Water	1 000 ml

6.4.2 *Preparation*

Dissolve 10 g of tryptose in 1 000 ml of water. Dispense the medium in 5 ml amounts into sterilized tubes and autoclave at 121 °C for 15 min.

6.5 Tryptose agar

6.5.1 *Composition*

Tryptose	10 g
Glucose	1 g
Sodium chloride	5 g
Agar	15 g
Thiamine hydrochloride	0.005 g
Water	1 000 ml

6.5.2 *Preparation*

Add ingredients to 1 000 ml of water, heat to boiling to obtain complete solution, cool to 50 °C to 60 °C, and adjust reaction so that the pH after sterilization will be 6.9 to 7.0. Distribute in bottles or tubes as required and sterilize in the autoclave at 121 °C for 15 minutes.

6.6 Azide dextrose broth

6.6.1 *Composition*

Polypeptone or peptone	15.0 g
Beef extract	4.5 g
Dextrose	7.5 g
Sodium chloride	7.5 g
Sodium azide	0.2 g
Water	1 000 ml

6.6.2 *Preparation*

Dissolve the ingredients in 1 000 ml of water. Heat with frequent stirring if necessary to obtain solution. Dispense in 10-ml volumes in approximate size test tubes. Sterilize by autoclaving at 118 °C for 15 minutes. Final pH should be 7.2 ± 0.2.

6.7 Hydrogen peroxide 25 per cent to 30 per cent aqueous.

6.8 Gram's stain

6.8.1 *Crystal violet solution*

6.8.1.1 Composition

Crystal violet (85 per cent to 90 per cent dye content)	2 g
Ethyl alcohol (95 per cent)	20 ml
Ammonium oxalate	0.8 g
Water	80 ml

6.8.1.2 Preparation

Dissolve the crystal violet in the alcohol and the ammonium oxalate in the water. Mix the two solutions and store the mixture for 24 h before use.

6.8.2 *Iodine solution*

6.8.2.1 Composition

Iodine	1 g
Potassium iodide	2 g
Water	100 ml

6.8.2.2 Preparation

Grind the potassium iodide and iodine together in a mortar adding small increments of water while grinding. Rinse the resulting solution into a volumetric flask and bring the volume to 100 ml.

6.8.3 *Counter stain*

6.8.3.1 Composition

Safranin	0.25 g
Ethyl alcohol	10 g
Water	100 ml

6.8.3.2 Preparation

Dissolve the safranin in the ethyl alcohol and mix the resultant solution with the water.

7 PROCEDURE

7.1 Plate count method

This method is suitable for analysing foods in which large numbers of *faecal streptococci* may be expected.

7.1.1 *Examination for presumptive faecal streptococci*

7.1.1.1 Prepare serial dilutions of food sample as described in Clause 7 of SLS 516 Part 1.

7.1.1.2 Transfer duplicate 0.1 ml aliquotes of a suitable dilution series on to the surface of dried plates of medium described in 6.3.

7.1.1.3 Spread each inoculum evenly over the entire agar surface using sterile bent glass rods of the *hockey stick* type.

7.1.1.4 Incubate the plates for 18 h to 24 h at 37 ± 1 ^{o}C.

7.1.1.5 Count the number of colonies sorrounded by black haloes if possible in plates containing between 20 and 1 000 of such colonies.

7.1.1.6 Calculate the presumptive number of colony forming units of *faecal streptococci* per 1 ml or 1 g by multiplying the average number of such colonies by ten times the dilution factor of the dilution which was counted.

7.1.2 *Confirmation of faecal streptococci*

7.1.2.1 Select some typical colonies as described in 7.1.1.5 strictly at random, such that the number is equal to the square root of the total.

7.1.2.2 Inoculate well isolated colonies into tubes of tryptose broth (6.4).

7.1.2.3 Incubate the tubes (7.1.2.2)at 45 ± 0.1 ^{o}C in a water bath for 18 h to 24 h.

7.1.2.4 Sub-culture the positive tubes onto Tryptose agar slants (6.5).

7.1.2.5 Incubate at 36 ± 1 °C overnight.

7.1.2.6 Prepare a smear of each culture and stain by Gram's method to confirm the presence of gram positive cocci.

7.1.2.7 Pipette sufficient hydrogen peroxide solution into the tubes (7.1.2.5) to cover the growth on slants.

7.1.2.8 Hold tubes at eye level and observe. The evolution of bubbles indicate a positive test.

7.1.2.9 Cultures which are gram positive, usually in chains of 2 cells, catalase negative and grow in tryptose broth at 45 ± 0.1 °C are considered faecal streptococci.

7.1.3 *Reporting of results*

7.1.3.1 Report the results as confirmed faecal streptococci per gram or millilitre of the product.

7.2 MPN method

This method is recommended for use in the routine surveillance of food for sanitary quality, in which small numbers of *faecal streptococci* may be expected.

7.2.1 Pipette 1 ml of each of all appropriate dilutions of the food homogenate (7.1.1.1) into each of 3 separate tubes of azide dextrose broth (6.6).

NOTE - If very low numbers of the organism are expected, 10 ml volumes of the lowest dilution may be added to equal volumes of double strength medium.

7.2.2 Incubate tubes at 36 ± 1 °C for 24 h and 48 h.

7.2.3 Observe the tubes at 24 h and 48 h for turbidity (growth).

7.2.4 Tubes showing turbidity are considered positive for faecal streptococci.

7.2.5 Determine the MPN of faecal streptococci per gram as in Clause 7.1.6 of SLS 516:Part 3.

7.3 Reporting of results

7.3.1 Report the results as the most probable number of *faecal streptococci* per gram or millilitre of the product.

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 results.

The report shall include all details required for complete identification of the sample.

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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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