SRI LANKA STANDARD 516 PART 14 : 2015

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METHODS OF TEST FOR MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS

PART 14 –EXAMINATION FOR SPECIFIC ORGANISMS-COLIFORMS AND *Escherichia coli* BY THE TRIPLICATE TUBE DETECTION METHOD

SRI LANKA STANDARDS INSTITUTION

Sri Lanka Standard

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SLS 516 Part 14 : 2015

Gr. 5

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Sri Lanka Standard

METHODS OF TEST FOR MICROBIOLOGY OF FOOD AND ANIMAL FEEDING STUFFS

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FOREWORD

This Sri Lanka Standard was approved by the Sectoral Committee on Food Products and was authorized for adoption and publication as a Sri Lanka Standard by the Council of the Sri Lanka Standards Institution on 2015-10-08.

In order to accommodate large number of test methods within the scope of one standard, this standard is published in several parts.

This method may be used when quantitative information about the number of organisms is not required.

The terms 'normative' and 'informative' have been used in the standard to define the application of the appendix to which they apply. A ' normative' appendix is an integral part of the standard, where as 'informative' appendix is only for information and guidance.

In the preparation of this standard the valuable assistance gained from the Australian Standard AS 5013.9, published by Standards Australia Limited is gratefully acknowledged.

1 SCOPE

This Standard describes the method for the examination of foods for coliforms and *Escherichia coli* by the triplicate tube method. This is a qualitative test and is suitable for determining the presence or absence of coliforms and *E.coli* in a stated quantity of material under test.

2 REFERENCES

Compendium of methods for the microbiological examination of foods, American Public Health Association, 2nd edition, 1984

SLS 393 Code of practice for preparation of test samples, initial suspension and decimal dilutions for microbiological examination of food and animal feeding stuffs
Part 1 General rules for the preparation of the initial suspensions and decimal dilutions

SLS 516 Methods of test for microbiology of food and animal feeding stuffs
Part 3 Horizontal Method for the detection and enumeration of coliforms, Section 1 Most probable number technique
Part 12 Horizontal method for the detection and enumeration of presumptive *Escherichia coli* - Most probable number technique

SLS 1461 Microbiological test methods for water
Part 1 Detection and enumeration of *Escherichia coli* and coliform bacteria, Section1 Membrane filtration method

SLS 1463 General requirements and guidance for microbiological examinations of food and animal feeding stuffs

3 DEFINITIONS

3.1 coliforms : Bacteria which at 36 ± 1 ^{o}C cause fermentation of lactose with the production of acid and gas in the operational conditions described.

3.2 *Escherichia coli* (*E. coli*) : Faecal coliforms which produce typical colonies on selective media which on sub-culture into peptone water produce indole at 44 ± 0.1 ^{o}C.

4 APPARATUS AND GLASSWARE

Usual microbiological laboratory equipment and, in particular the following shall be used:

4.1 *Apparatus for dry sterilization (oven) or wet sterilization (autoclave)*

4.2 *Incubator,* capable of operating 30 ± 1^{o}C or 37 ± 1^{o}C

4.3 *Water bath,* capable of operating between 44.0 ^{o}C to 44.5 ^{o}C

4.4 *Test tubes,* of dimensions approximately 16 mm x 160 mm

4.5 *Petri dishes*, glass or plastic diameter 90 mm or 100 mm

4.6 *Durham fermentation tubes*, for fitting to 16 mm x 160 mm test tubes

4.7 *Loops,* of platinum –irridium or nickel-chromium, diameter approximately 3 mm

4.8 *Total delivery pipettes*, having nominal capacities of 1 ml and 10 ml

4.9 *pH meter*, accurate to within ± 0.1 pH units at 25 ^{o}C

5 CULTURE MEDIA, DILUENTS AND REAGENTS

5.1 Culture media and reagents (see Appendix **A)**

5.1.1 *EC broth*

5.1.2 *Eosin methylene blue (EMB) agar*

5.1.3 *Indole reagent (Kovac's)*

5.1.4 *Lauryl tryptose (LT) broth*

5.1.5 *Tryptone water*

5.2 Reference cultures

5.2.1 *Enterobacter aerogenes*

NCTC 10006 or ATCC 13048 or UQM 1976 – this organism gives positive reactions for coliforms and negative reactions for *E. coli.*

5.2.2 *Escherichia coli*

NCTC 9001 or ATCC 11775 or UQM 1803 – this organism gives positive reactions for coliforms and *E.coli.*

NOTE
The reference cultures are used as instructed in these methods. They are subjected to the test procedures at the same time as test samples in order to demonstrate that appropriate positive and negative reactions are obtained in the tests. A test shall be regarded as invalid unless the reference cultures give appropriate results.

6. PROCEDURE

6.1 Presumptive test for coliforms

6.1.1 Prepare the sample for microbiological examination in accordance with the instruction given in the relevant **SLS 393** methods for the examination of specific product.

6.1.2 Using the technique described in **SLS 393** Part **1**, prepare dilutions, as required, of the sample.

6.1.3 Inoculate 1 ml of the appropriate dilution to be examined, or, where appropriate, 1 ml of the original sample, into each of three tubes containing 10 ml of LT broth **(5.1.4**).

NOTE
The amount of material inoculated into each tube is calculated using the volume (1 ml) and the dilution used.

6.1.4 If the test is for coliforms, prepare a control tube by inoculating 10 ml of LT broth (**5.1.4**) with either of the reference cultures (**5.2.1** or **5.2.2**).

If testing for *E. coli* prepare control tubes of each reference culture by inoculating two 10 ml portions of LT broth (**5.1.4**) respectively with the two reference cultures.

6.1.5 Mix the tubes by gentle rotation.

6.1.6 Incubate at 30 ± 1 ^{o}C or 37 ± 1 ^{o}C (see Note 3) for up to 48 h, and examine for gas production after 24 h and 48 h. Approximately 30 min before each examination, gently tap all tubes to guard against false negative results due to gas supersaturation.

6.1.7 A presumptive positive reaction is indicated by the production of sufficient gas to fill the concavity of the Durham tube.

6.1.8 Record the result of the presumptive test as positive if at least two of the set of three tubes are positive.

NOTES
*1) A flow diagram of the procedures is shown in Appendix **B**.*
2) The method describes a general method and modifications may be required according to the material under test.
3) Where the test is to estimate the level of coliforms only as an index of potential spoilage of the product and of hygiene, an incubation temperature of 30 ^{o}C is specified. However, where the test is to be extended to include the estimation or detection of E.coli, incubation at 37 ^{o}C is specified. Cultures which have been incubated initially at 30 ^{o}C cannot be used for further tests for E. coli.
*4) Methods for the examination of rinse fluids and filterable liquid products for coliforms and E.coli by membrane filtration are given in **SLS 1461** Part **1**/Section **1***
*5) Quantitative coliform and E.coli methods are given in **SLS 516** Parts **3** and **12** respectively.*

6.2 Confirmatory test for coliforms

6.2.1 Subculture all cultures as they become positive in the presumptive test by streaking onto plates of EMB agar (**5.1.2**) and incubate at 30 ± 1 ^{o}C or 37 ± 1 ^{o}C for up to 18 h to 24h.

6.2.2 Colonies of typical coliform appearance shall be regarded as coliforms.

NOTES
Typical coliform colonies may show any of the following characteristics:
i) Green metallic sheen.
ii) Dark purple centres.
iii) Opaque, unnucleated , mucoid and pink coloration.

6.2.3 Record the number of tubes from each dilution that yields plates containing typical coliform colonies.

6.3 Tests for *E. coli*

6.3.1 Using a straight wire, subculture all cultures as they become positive at 24 h and at 48 h (see Clause **6.1.6**) in to EC broth (**5.1.1**) previously warmed to approximately 44 ^{o}C.

6.3.2 Incubate in a water bath at 44.0 ^{o}C to 44.5 ^{o}C for up to 48 h, together with the positive and negative reference cultures, and examine at 24 h and 48 h. Tap the tubes gently after the initial 24 h period (see Clause **6.1.6**). Those tubes showing gas production shall be deemed to be presumptively positive.

6.3.3 Inoculate an EMB agar (**5.1.2**) plate to obtain single colonies from each positive test obtained.

6.3.4 Incubate at 37 ± 1 ^{o}C for up to 18 h to 24 h.

6.3.5 Select up to three typical colonies showing a green metallic sheen by reflected light or dark purple centres by transmitted light, or both from each plate. Subculture into individual tubes of tryptone water **(5.1.5)** and incubate at 44.0 ^{o}C to 44.5 ^{o}C for 24 ± 1 h.

6.3.6 Test the cultures for indole production by using Kovac's indole reagent (**5.1.3**). Tubes indicating the presence of indole are recorded as positive for the presence of *E. coli*.

6.3.7 Record the number of positive tubes.

6.4 Optional confirmatory tests

Where further confirmation is required, the methyl red, Voges –Proskauer and citrate tests as described in Compendium of methods for the microbiological examination of foods, are recommended.

6.5 Interpretation of results

Record the presence of coliforms and *E.coli* in the amount of material inoculated into each tube, provided that confirmed organisms are recorded in at least two of the three tubes.

7 TEST REPORT

7.1 The test report shall include all information necessary for the complete identification of the sample.

7.2 The presence or absence of coliforms or *E. coli* in the amount of material inoculated into each tube, stating whether organisms present are presumptive or confirmed. When reporting coliforms, state the incubation temperature used.

APPENDIX A
CULTURE MEDIA
(Normative)

B. 1 EC broth

B.1.1 Composition

Tryptone or trypticase	20.0 g
Lactose	5.0 g
Bile salts No. 3	1.5 g
Potassium monohydrogen phosphate (K_2HPO_4)	4.0 g
Potassum dihydrogen phosphate (KH_2PO_4)	1.5 g
Sodium chloride	5.0 g
Distilled Water	to make 1000 ml

B.1.2 Preparation

Dissolve ingredients, make up to 1 liter and dispense into suitable tubes, containing fermentation tubes in sufficient quantities to cover the inverted fermentation tubes after sterilization for 15 min in an autoclave set at 121 ^{o}C.
Adjust the pH, if necessary so that after sterilization it is 6.9 ±0.1 at 25 ^{o}C.

B. 2 Eosine Methylene Blue (EMB) agar

B.2.1 Composition

Peptone	10.0 g
Lactose	10.0 g
Potassium monohydrogen phosphate (K_2HPO_4)	2.0 g
Eosin Y, 20 g/l aqueous solution	20.0 ml
Methylene blue, 2.5 g/l aqueous solution	25.0 ml
Agar	15.0 g
Water	to make 1000 ml

B.2.2 Preparation

Add all ingredients to water, heat to boiling to obtain complete solution, cool to 50 ^{o}C to 60 ^{o}C, mix well and adjust pH so that final pH will be 7.1 ±1. Dispense as required and autoclave at 121 ^{o}C for 15 min.

B.3 Indole reagent (Kovacs)

B.3.1 Composition

p-Dimethylaminobenzaldehyde	10.0 g
Amyl (or isoamyl) alcohol	150 ml
Conc. Hydrochloric acid	50 ml

B.3.2 Preparation

Dissolve *p*-Dimethylaminobenzaldehyde in alcohol. Gently heat (50 ^{o}C to 55 ^{o}C). Cool and then slowly add the concentrated hydrochloric acid. The final solution shall be stored in the refrigerator and protected from light.

NOTE
The reagent shall be light yellow to brown in colour. Some brands of amyl alcohol produce a dark- coloured reagent which is unsatisfactory.

B.4 Lauryl tryptose (LT) broth

B.4.1 Composition

Tryptose	20.0 g
Lactose	5.0 g
Potassium monohydrogen phosphate (K_2HPO_4)	2.75 g
Potassium dihydrogen phosphate (KH_2PO_4)	2.75 g
Sodium chloride	5.0 g
Sodium lauryl sulfate	0.1 g
Water	1000 ml

B. 4.2 Preparation

Dissolve the ingredients in water. Dispense in tubes with inverted fermentation tubes so that liquid covers the inverted fermentation tube. Autoclave at 121 ^{o}C for 15 min.

B.5 Tryptone water

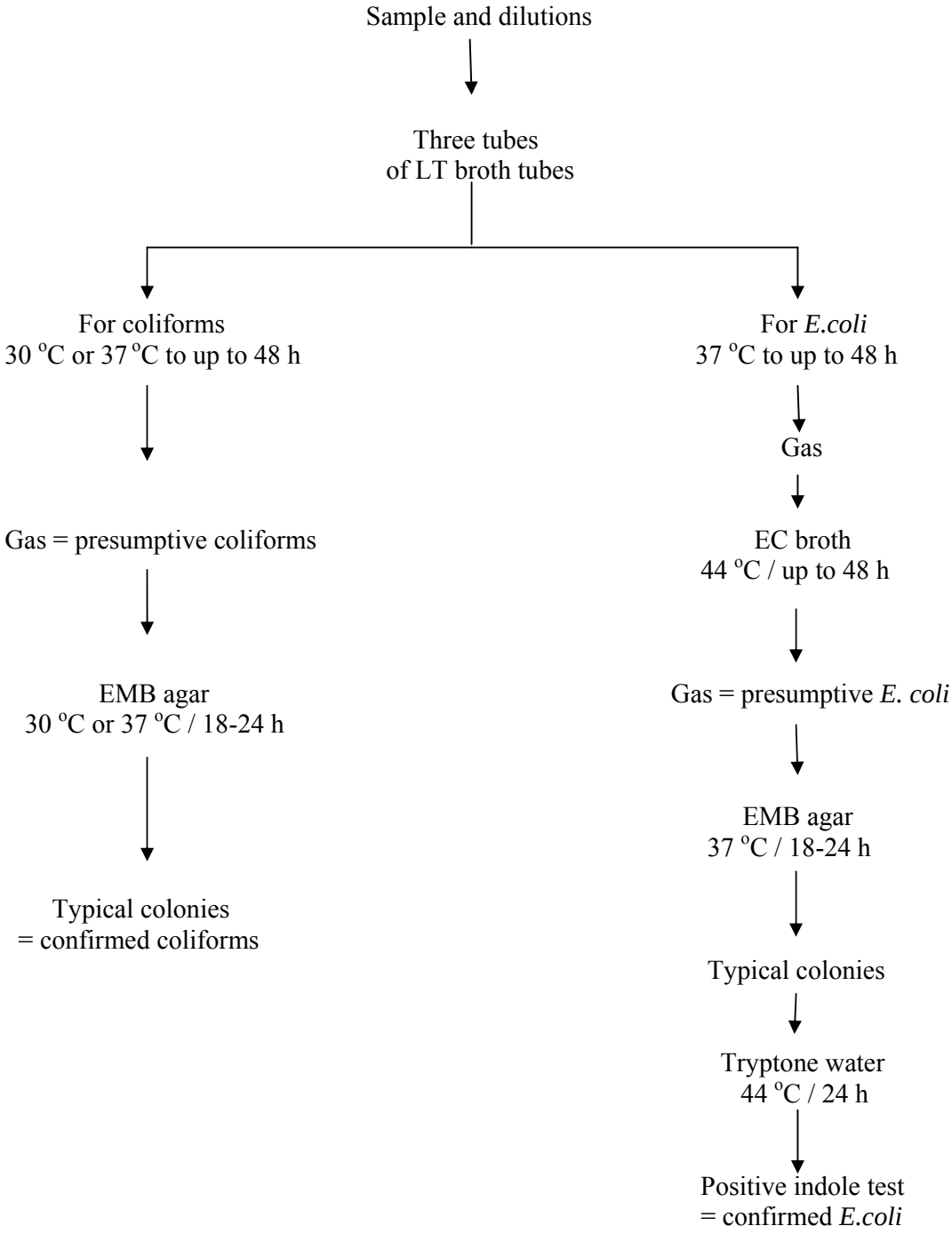
B.5.1 Composition

Tryptone	10.0 g
Sodium chloride	5.0 g
Water	1000 ml

B. 5.2 Preparation

Dissolve the ingredients in the water and adjust pH so that after sterilization it is 7.5 ±0.1. Dispense as required and autoclave at 121 ^{o}C for 15 min.

APPENDIX B
FLOW DIAGRAM OF TESTS FOR COLIFORMS AND *Escherichia coli*
(Informative)



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