COLIFORMS AND ESCHERICHIA COLI

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INTRODUCTION

Coliforms are Gram-negative, non-sporing, facultatively anaerobic rods which ferment lactose, producing acid and gas within 48 hrs and they belong to the family Enterobacteriaceae. The coliform group includes several genera, some of which are of intestinal origin (Escherichia) while others are associated with plant and soil material (Enterobacter). Thus it is actually a misconception to consider the coliform group as simply an indicator of faecal pollution.

However, generally speaking, it is the count of E. coli that is a more reliable indicator of faecal contamination. Its presence indicates recent faecal contamination as it generally does not survive for long in environments other than the intestine.

Faecal coliforms are a group of coliforms capable of fermenting lactose to produce acid and gas at both 37°C and 44.5 ± 0.5°C in 48 hrs and generally contain a high proportion of E. coli. As a significant number of non-faecal coliforms can give a positive faecal coliform test, the test can be made more specific for E. coli by testing for the production of indole at 44.5 ± 0.5°C.

I CULTURE MEDIA*

Brilliant green bile broth (BGB)
Butterfield's buffered phosphate diluent
Eosin methylene blue agar (EMB)
Koser citrate medium
Lauryl sulphate tryptose broth (LST) or EC medium
MRVP medium
SIM medium
Simmons citrate agar
Nutrient broth

* Refer to Appendix B for methods of media preparation.

II CHEMICAL REAGENTS**

a) Kovac's reagent

b) Methyl red solution

c) α-naphthol solution (5% w/v)

d) KOH SOLUTION (40% w/v)

** Refer to Appendix D for methods of reagent preparation.

III APPARATUS

'Waring' blender & flask	Autoclave
Pipettes	Incubator
Scissors & forceps	Water-bath
Alcohol lamps	Weighing balance
Alcohol (70% v/v) swabs	Laminar flow chamber

IV SAMPLING PROCEDURE

Refer to "AEROBIC PLATE COUNT" (E-2) SECTION III

V SAMPLE PREPARATIONS

Refer to "AEROBIC PLATE COUNT' (E-2) SECTION IV

VI PROCEDURE

A. EXAMINATION FOR PRESUMPTIVE COLIFORMS

1. Select appropriate dilutions and for every dilution, transfer 1 ml aliquots into each of 3 LST tubes.

2. Invert tubes to ensure Durham tubes do not contain gas bubbles.

3. Incubate the tubes at 35°C for 48 hrs.

4. Any tube producing gas is considered positive for the presence of coliforms.

B. CONFIRMATION TESTS FOR COLIFORMS

1. Transfer a loopful of suspension from a positive LST tube into a tube of BGB broth.

2. Invert tubes to ensure Durham tubes do not contain gas bubbles.

3. Incubate the BGB tubes at 35°C for 48 hrs.

4. Examine for gas production.

5. Using the MPN Tables (Appendix A), calculate the MPN of coliforms based on the proportion of confirmed LST tubes (with gas production) for 3 consecutive dilutions.

C. EXAMINATION FOR PRESUMPTIVE E. COLI

1. Transfer a loopful from each LST tube (with gas production) into a tube of EC medium prewarmed to 44.5°C.

2. Incubate the BGB tubes at 44.5°C for 48 hrs.

3. Examine for gas production at 24 hrs and, if negative, again at 48 hrs.

4. Any tube showing gas production is considered positive for the presence of presumptive E. coli.

D. CONFIRMATION TESTS FOR E. COLI

1. Subculture all positive EC tubes by streaking onto plates of EMB agar.

2. Incubate at 35°C for 18-24 hrs.

3. Examine the plates for suspicious E. coli colonies, ie. black or dark centred with or without the greenish metallic sheen.

4. Subculture the suspected E. coli colonies in nutrient broth and incubate at 35°C for 18-24 hrs.

5. Perform the following biochemical tests*:

   Indole production

   Methyl-Red & Voges-Proskauer tests

   Citrate utilization

   * Refer to Appendix C for biochemical tests procedures.

6. Interpret results as follow:

Indole	MR	VP	Citrate	Type
+	+	–	–	Typical *E. coli*
–	+	–	–	Atypical *E. coli*
+	+	–	+	Typical Intermediate
–	+	–	+	Atypical Intermediate
–	–	+	+	Typical *Enterobacter aerogenes*
+	–	+	+	Atypical *Enterobacter aerogenes*

Other groupings may appear : in such cases cultures are usually mixed. Restreak to det. their purity.

Compute MPN of *E. coli*/g, considering Gram neg., nonspore-forming rods producing gas in lactose and producing ++-- or -+-- IMViC patterns as *E. coli*.

Refs. : JAOAC 49 : 270, 276 (1966); 51 : 865, 867 (1968); 58 : 1154 (1975).

VII CALCULATION OF MPN

Most Probable Number = Index/g
See Appendix A and A1

REFERENCES

Hazzard. (1985). ASEAN Training Course in Fish Quality Control. Training course organised by HAWKAID, Hawkesbury Agricultural College Research and Development Co. Ltd.
Chapter : Microbiology in Seafood Quality Control, Section 2:16.
Chapter : Fish quality control microbiology Section 6:88.

Official Methods of Analysis, Association of Official Analytical Chemists, 14th Edition, 1984.

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR COLIFORMS AND E. COLI