

Department	Micro Laboratory	Document no	MICLAB – METHOD 004		
Title	Presence or Absence Test of E.Coli				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1. SCOPE AND APPLICATION

This General Test Method shall be followed in order to determine the presence or absence of E. coli. This procedure applies to all products manufactured at the GMP site and the raw materials used to manufacture those products.

2. REAGENTS AND MATERIALS REQUIRED

- 2.1 Lauryl Tryptone Broth (LTB) (OXOID CM451) in test tubes containing Durham tubes.
- 2.2 Double Strength Lauryl Tryptone Broth (D/S LTB) in test tubes containing Durham tubes.
- 2.3 E.C. Broth (OXOID CM853) - in test tubes containing Durham tube.
- 2.4 Eosin Methylene Blue (EMB) (OXOID CM69) agar.
- 2.5 Tryptone Water (TW) – (OXOID CM87)
- 2.6 Indole Reagent (KOVAC's).
- 2.7 0.1% Peptone or other appropriate diluent.
- 2.8 Thermometer with 0.1°C graduations.

3. GENERAL TEST METHOD

- 3.1 For the presence or absence of E.coli/g weigh out 10g of sample into a stomacher bag.
- 3.2 Make up to 100g with 0.1% Peptone or chosen diluent. Mix well.
- 3.3 Inoculate 10mL of the above dilution into each of 3 tubes containing 10mLs of D/S LTB. If a higher sensitivity is expected inoculate an appropriate amount of the above dilution (ie. 1mL and 0.1mL) into 3 tubes containing 10 mLs of LTB.
- 3.4 Inoculate 2 additional tubes with reference cultures.

Negative control -	<u>Enterobacter aerogenes</u> NCTC 10006 or ATCC 13048
Positive control -	<u>E. coli</u> NCTC 9001 or ATCC 1175
- 3.5 Mix the tubes by gentle rotation.
- 3.6 Incubate the inoculated tubes of 37 ± 1°C for up to 48 hours and examine for gas production after 24 hours and 48 hours.

Note: Approximately 30 minutes before each examination, gently tap all tubes to guard against false negative results due to gas supersaturation.
- 3.7 A tube is regarded as positive if sufficient gas is produced to fill the concavity of the Durham tube.

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Note: If none or only one of the 3 tubes shows a positive reaction record the results as negative. If at least 2 of the tubes show gas production, proceed with the remainder of the test.

3.8 Using a sterile inoculating loop, subculture all cultures as they become positive at 24 hours and 48 hours into EC broth previously warmed to approximately 37°C; include both reference cultures.

3.9 Incubate in a water bath at 44.0°C to 44.5°C for up to 48 hour and examine at 24 hour and 48 hour. Tap the tubes gently after the initial 24 hour period. Those tubes showing gas production shall be deemed to be positive.

Note: Thermometer with 0.1°C graduations must be used to check water bath temperature.

3.10 Inoculate a dried EMB agar plate to obtain single colonies from each positive test obtained, and from both reference cultures.

3.11 Incubate the EMB plate at 37 ± 1°C for 18 hours to 24 hours.

3.12 Select at least three typical colonies showing a green metallic sheen by reflected light and / or dark purple centres by transmitted light, from each plate, and one typical colony of each reference culture. Subculture into individual tubes of Tryptone Water (for Indole test) and incubate at 44.0°C to 44.5°C for 24 hours.

3.13 Test the cultures for indole production by using Kovac's indole reagent. Add 1 drop into each tube. Tubes indicating the presence of indole (appearance of a pink ring) are recorded as positive for the presence of E. coli.

3.14 Record the number of positive tubes.

3.15 Calculation and report.

Record presence or absence of E. coli in the amount inoculated into each tube, provided that confirmed organisms are recorded in at least two of the three tubes. Report the method used and any circumstances which may have influenced results of the test.

3.16 Optional confirmatory tests.

Where further confirmation is required, the methyl red, Voges-Proskauer and citrate tests are recommended (Refer to Compendium of Methods for the Microbiological Examination of Foods - 2nd Edition 1984 American Public Health Association).

Note: Voges-Proskauer and citrate tests can be determined using Biomerieux API20E strip.

4. REFERENCE DOCUMENTS

4.1 Speck M.L. Compendium of Methods for the Microbiological Examination of Foods (2nd edition 1984). American Public Health Association - Washington.

5. REVIEW HISTORY

Version #	Revision History
MICLAB – METHOD 004	New