

Department	Micro Laboratory	Document no	MICLAB – METHOD 012		
Title	Presence or Absence Test of Clostridium Perfringens				
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 *Clostridium perfringens* in raw materials and finished products.

2. REAGENTS AND MATERIALS REQUIRED

- 2.1 Cooked Meat Medium (CMM) (OXOID CM81)
- 2.2 Thioglycollate + 0.5% Tween 80 (THIO+T) (OXOID CM173)
- 2.3 Tryptose Sulfite Cycloserine (TSC) agar (OXOID CM 587, SR88, SR47)
- 2.4 Streaking loops - 10µL
- 2.5 Solution A - Sulfanilic Acid
- 2.6 Solution B - N-(1-naphthyl) ethylenediamine reagent
- 2.7 Lactose – Gelatin (LG) Medium
- 2.8 Nitrate Motility (NM) Medium
- 2.9 Reference culture *C. perfringens* N.C.T.C. 8237
- 2.10 Tryptone Soya Agar (TSA)
- 2.11 Lactose Egg Yolk (LEY) Agar

3. GENERAL TEST PROCEDURE

- 3.1 Weigh out 1.0g of sample into CMM or THIO+T as pre-determined by validation study.

Note: If CMM is not freshly made, or if THIO+T as oxidised, boil medium for 10 min. to drive out oxygen. Cool down media in water bath to approximately 45-46°C.

Carry out a positive control by inoculating a 10µL loopful of *C. perfringens* into the appropriate enrichment broth.

- 3.2 Incubate at 37 ± 1°C for 48 hours.
- 3.3 Streak a 10µL loop full of enrichment and control onto a dried TSC agar plate.
- 3.4 When the inoculum has been absorbed into the agar, overlay plates with an additional 10 ml of TSC agar without egg yolk.
Allow overlay to set.
- 3.5 Incubate plates anaerobically at 37 ± 1°C for 18-24 hours.
- 3.6 Examine plates for black colonies surrounded by opaque white haloes due to lecithinase activity.

NOTE: Routinely confirm suspect colonies by streaking onto 2 TSA plates and incubating one aerobically and one anaerobically. Typical colonies for *C. perfringens* will not

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grow aerobically. If only anaerobic growth is found set up a RAPID ID32A identification strip and follow the manufacturers instructions for anaerobic microorganisms.

The following describes the confirmatory steps outlined by the Australian Standards AS 1766.2,8 - 1991.

3.7 Confirmation Tests:

- Subculture suspect colonies onto LEY agar (lactose egg yolk), incubate anaerobically for 24 hours at 37 ± 1°C. Check for purity.
- Examine culture by Gram stain. Gram positive - short, thick rods, occurring singularly or in pairs or as short chains.
- Inoculate tubes LG medium (Lactose Gelatin medium) NM (Nitrate Motility medium) by stabbing with a straight wire.
- Incubate at 37 ± 1°C. for 24 hours.

3.8 Examine LG Medium.

- Lactose fermentation - gas production and colour change from red to yellow.
- Gelatin liquefaction - chill tubes at 5°C. for 1 hour. If the medium gel, incubate at 37 ± 1°C. for another 24 hours and recheck for gelatin liquefaction.
C. perfringens - ferments lactose and liquefies gelatin.

3.9 Examine NM Medium.

Motility - diffuse growth out into the medium away from the stab line.

Nitrate detection - add 0.5 ml of solution A and 0.2 ml of Solution B.

Development of red colour indicates presence of nitrites. If no colour develops, test for residual nitrate by adding powdered zinc. If red colour does not develop after addition of zinc the organism does not reduce nitrate to nitrite.

C. perfringens is non-motile - reduces nitrate to nitrite.

4. REFERENCE DOCUMENTS

- 4.1 The Microbiological Update for Pharmaceuticals, Medical Devices and Cosmetics Vol.18 No.11 February 2001 Editor, Murray S Cooper, Phd.
- 4.2 USP 24-NF 19 Supplement 2

5. REVIEW HISTORY

Version #	Revision History
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