Processing Winter School on Advances in microbiological and biotechnological methods for the detection of pathogenic microorganisms and their toxins in fishery environments,organized by CIFT at Cochin,5-25 November:87,2001

# P7: Enumeration, Isolation and Identification of *Clostridium perfringens*

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The method for enumeration and isolation of *Clostridium perfringens* employs selective enumeration agars incorporating iron and sulphite ions, which allow sulphite reducing Clostridia to form black colonies

## Procedure

1. Aseptically weigh 25g fish sample into a sterile stomacher bag and add 225ml peptone dilution fluid. Blend for I min at low speed.
2. Make serial dilutions from $10^1$ to $10^5$. Mix each dilution before each transfer.
3. Pour 6-7 ml Tryptose Sulphite Cycloserine (TSC) agar into each of six 100x15 mm petridishes and spread evenly on bottom and allow to solidify. Label the plates.
4. Aseptically transfer I ml of each dilution of homogenate to the centre of duplicate sets of agar plates.
5. Pour additional 15 ml TSC agar into dish and mix with inoculum by gently rotating dish and allow to solidify.
6. Place the plates in upright position in anaerobic jar (Gas Pak jar), establish anaerobic conditions and place jar in 37°C incubator for 20-24 h.
7. Remove the plates from anaerobic jar and count the black colonies.
8. Select typical *C. perfringens* colonies and inoculate into tubes of freshly deaerated and cooled fluid thioglycollate broth.
9. Incubate at 37°C for 18-24 h. Examine each culture by Gram stain and check for purity. If there is evidence of contamination, restreak onto TSC agar and isolate pure colonies.
10. Inoculate modified iron-milk medium with I ml of actively growing fluid thioglycollate culture and incubate at 46°C in a water bath. After 2 h, check hourly for stormy fermentation .
11. Inoculate Fluid Thioglycollate broth culture into: 1) Motility nitrate medium. 2) lactose gelatin media. 3) Raffinose sugar fermentation broth. 4) Salicin sugar fermentation broth.

The typical reaction of *C. perfringens* is as follows.

| Iron milk | Stormy clot |
|---|---|
| Motility | - |
| Nitrate | + |
| Lactose | + |
| Gelatin | + |
| Raffinose | + |
| Salicin | - |

12. Calculate number of *C. perfringens* cells /g fish on the basis of percent of colonies tested that are confirmed as *C. perfringens.*

Number of *C. perfringens* cells /g fish =

Total count x No. of isolates positive x dilution factor / Total no. of isolates

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