P6: Isolation of *Clostridium botulinum* from Fish/Shellfish

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Direct plating of contaminated samples without enrichment is seldom successful since *Clostridium botulinum* is normally outnumbered by other facultative and strictly anaerobic bacteria. Isolation is normally achieved, therefore, from an enrichment culture or an incubated food sample.

For isolation and characterization of *C. botulinum* from suspected food sample (fish/ shrimp), a procedure followed by USFDA is employed.

1. Aseptically transfer 2-5 g sample directly into enrichment broth (CMM/TPGYB)
2. Incubate at 28 ± 2°C for 5-6 days.
3. Two ml each of enrichment culture sediment is transferred to two sterile test tubes. Add equal volume filter sterilized absolute ethyl alcohol to 1 tube and hold at room temperature for 1 h with occasional shaking. Heat the other tube at 80°C for 10 min.
4. After pretreatment, streak enrichment cultures on to predried plates of TSGYA and incubate for 48-72h at 30°C under anaerobic conditions of Gas-Pak system.
5. Select *Clostridium botulinum* colonies from the plate. Typical *Clostridium botulinum* colonies are raised or flat and commonly show some spreading and exhibit surface irridescence (pearly layer) covering both the zone of precipitation and the halo of clearing around the colony as seen by reflected light. With sterile loop, transfer selected colonies into tubes of CMM or TPGYB and incubate at 30°C for 3 days.
6. To test these cultures for the presence of toxin, centrifuge a portion of the culture at 10000xg and 4°C for 15min and take the supernatant for toxicity test in mice. Toxicity is tested by mouse bioassay.

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