Revive from an L-dried ampoule of an anaerobic strain

This is to illustrate how strains of aerotolerant anaerobic bacteria preserved by the L-drying method (drying from a liquid-state) are re-suspended in liquid cultures at the anaerobic gassing station of JCM.



Prepare three anaerobic culture tubes (No. 1 to No. 3 tubs, 5-10 ml medium for each). Two disposal plastic syringes will be used (one with a 70 mm long needle).



Crack open the ampoule as usual. Flash the longneedle syringe several times with sterile, oxygen-free gas by drawing in and expelling gas.



With the long-needle syringe, remove 0.3 to 0.5 ml medium anaerobically from No. 1 tube to the bottom of the ampoule. Slowly suspend cells in liquid culture by drawing in and expelling the liquid medium without make bubbles.



Remove and inoculate the cell suspension into the medium of No. 1 tube carefully to avoid inoculating air.



Using another syringe flashed with sterile oxygen-free gas, transfer a certain amount of the cell-suspension (e.g., 0.5-1.0 ml) from No. 1 tube to No. 2 tube.



Likewise, transfer a certain amount of the cell-suspension from No. 2 tube to No. 3 tube. Incubated all the culture tubes at the designated temperature.