

## **Opening the vial and reviving culture for the strictly anaerobic bacteria**

Requirements for conducting these procedures in a sterile environment:

1. Notice that the strictly anaerobes were lyophilized by using broth-drying or embraced on glassbeads. The lyophilized cells always in the form of mist or powder not solid mass were investigated.
2. Sterilize the outside of the vial by wiping 70% alcohol. Heat the tip of the outer vial in the flame.
3. Squirt a few drops of water on the hot tip to crack glass. Strike with file to remove tip.
4. Remove the insulation and inner vial with forceps. Gently raise cotton plug.
5. Using a sterile Pasteur pipette, add 0.3 to 0.5 ml of suitable rehydration fluid into the vial. Pipetting several times to mix well.
6. Transfer the total mixture to a vial containing the recommended broth medium. Incubate cultures under the appropriate conditions for few days. The culture medium will become turbidity means the strain is growing.
7. **The culture medium should filled with carbon dioxide to keep the strictly anaerobic environment since opening the vial.**
8. If after a week of a culture is unsatisfactory, or having questions about a culture. Please notify BCRC **within a month** so that the strain in question can be investigated.
9. Store freeze-dried cultures at 4°C if they are not immediately rehydrated