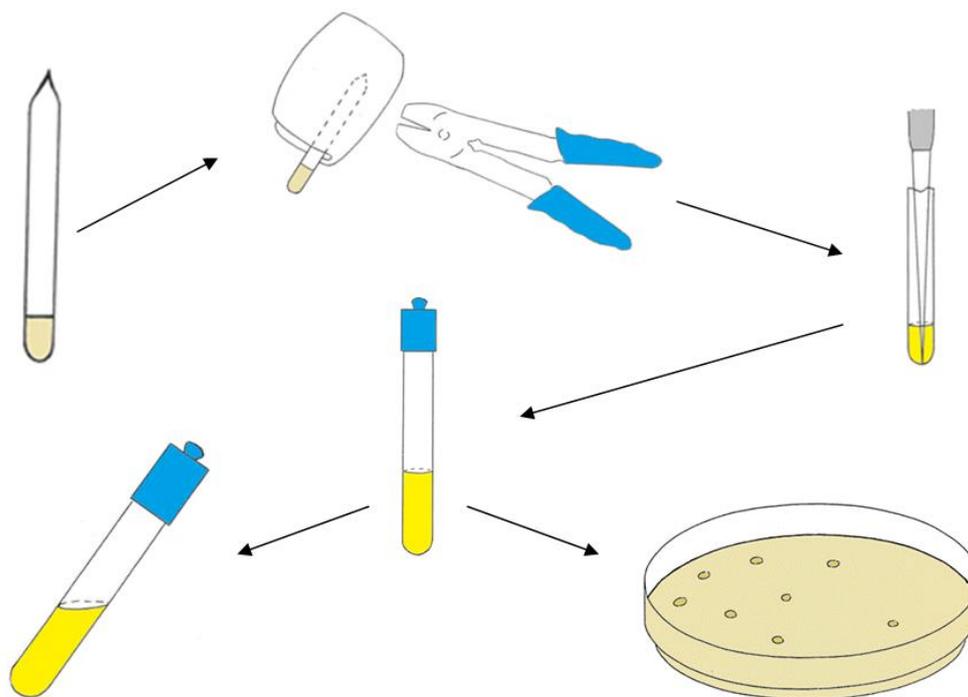




## REVIVAL OF FREEZE-DRIED CULTURES

The opening of ampoules with freeze-dried microorganisms, as well as subsequent handling with them, is carried out only in microbiologically safe environment. Ampoules with pathogens should be opened in an exhaust protective cabinet!

1. Take out the glass ampoule from the transport packaging. Remove the label and clean the ampoule with 70% ethanol or other disinfectant.
2. Wrap/insert the ampoule into a folded sterile gauze/"pocket" to cover 3/4 of its top edge.
3. Break the ampoule through the gauze with a sharp blow of an appropriate metal tool (thin pliers/ hammer, pre-treated with 70% ethanol). Make sure the breakage is at a distance of 1/3 from the top.
4. Carefully remove the ampoule from the gauze (without the broken glass) and place it on a suitable stand.
5. Add 0.2 ml of 0.1 % pepton water or other recommended broth to the contents of the ampoule in sterile conditions using a micropipette (automatic pipette or syringe). Glue the ampoule with a suitable sterile container.
6. Allow the culture to recover at room temperature for 30 minutes (with laminar flow on, or near a lit spirit lamp).



7. Homogenize the contents of the ampoule gently with a micropipette (automatic pipette or syringe) and transfer the suspension to a tube with a 3-5 ml broth recommended for the strain.
8. Homogenize the suspension in the tube by stirring with a pipette or a vibrating shaker. Avoid bubbles or foam formation, if the strain is anaerobic or microaerophilic.
9. Incubate the culture under the recommended for the strain conditions.
10. The obtained living culture can be transferred to suitable liquid/solid media depending on the purposes of the work.
11. All waste from the handling the ampoule is decontaminating by autoclaving at 1.5 atm (127°C) for 45+60 min.
12. Ampoules with freeze-dried microorganisms may be stored in a regular refrigerator (4-8°C).

*Note.* Most lyophilized cultures will remain viable for a long period when kept under optimal conditions. This period varies among different strains. Furthermore, some ampoules may not have been sealed perfectly or may crack during storage. NBIMCC recommended reviving the culture within a few months.