

## INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILIZED REFERENCE STRAIN CULTURES

Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available at <http://www.sci.muni.cz>.

Lyophilized cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

- A) Check the number of the culture on the label inside the ampoule
- B) Make a file cut on the ampoule near the middle of the plug (see Figure 1)
- C) Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
- D) Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
- E) Remove the pointed end of the ampoule into disinfectant
- F) Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
- G) Incubate the inoculated medium at appropriate conditions for several days
- H) Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

### Notes:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see <http://www.sci.muni.cz>)
- Cultures may need at least one sub-culturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

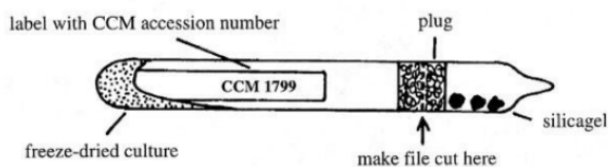


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on <http://www.sci.muni.cz>