

OPENING OF AMPOULES AND REHYDRATION OF FREEZE-DRIED STRAINS

NOTE: KEEP THE DOCUMENTS ACCOMPANYING THIS SHEET, YOU MAY NEED THE DATA WITHIN THEM LATER

Before you start...

1. Store conveniently the ampoules you may not use immediately

Keep them in the dark at mild temperature (at 4 to 24°C, preferably 18°C). Do not freeze them

Even though freeze-dried material can be preserved for very long periods, viability losses can occur. Hence, it is advised that rehydration is scheduled upon reception of the ampoules or at latest within one year

2. Check the availability of the recommended media

Consult the delivery note or our on line catalogue (www.cect.org) for recommended media

Please note that usually both the broth and the solid medium (with agar) are required

Media have to be in good use (not too dry or too wet, sterile ...)

3. Check that you can control other incubation parameters

Again, consult the delivery note or our on line catalogue (www.cect.org) for recommended temperatures, atmosphere ...

To work with anaerobic or microaerophilic organisms various strategies can be followed. Consult specialized literature if you are not familiar with such procedures

Opening of ampoules

1. Check list

In addition to the items mentioned above you will need the following: broken glass disposal, sterile water, Pasteur pipettes and forceps

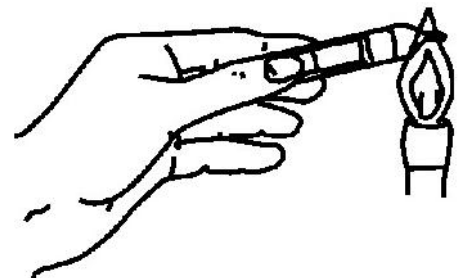
Work in microbiologically controlled conditions

2. Heat the tip of the ampoule with a flame

Usually only 5-15 seconds suffice (though it may take longer if the flame is too weak)

Make sure that only the tip is being heated in order to prevent a loss of viability of the preserved material

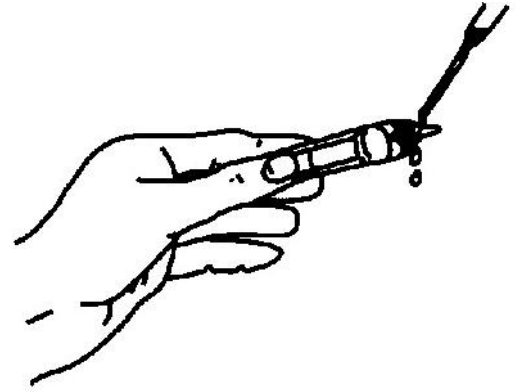
The inner cotton plug should not become brown (this indicates excessive heating)



3. Break the tip of the ampoule

Place 1-4 drops of sterile water onto the hot tip to crack the glass

If no breakage occurs, repeat the previous step trying to increase the heating of the tip (however excessive heating is not desirable either since it may damage the strain)



4. Remove glass fragments

Wear protective goggles if you think that small glass fragments may reach your eyes (for instance if you are not working at a vertical flow bench)

Carefully strike off the glass tip with an appropriate tool (e. g. forceps)

Lift the cotton plug with the forceps so that it can be more easily handled in coming steps

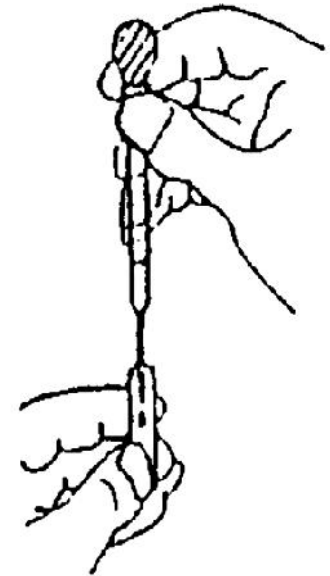


Rehydration of strain and inoculation

1. Rehydration

Add 0.2-0.3 ml of recommended broth using a Pasteur pipette

Carefully suspend the cells until the appearance of the liquid is homogeneous. For better mixing the suspension can be gently loaded into the pipette several times trying not to form air bubbles or foam



2. Inoculation

Use several drops of the suspension to inoculate an agar Petri plate and/or a slant and transfer the rest to a tube with 5-10 ml of the recommended liquid medium. Some cells may show a prolonged lag period; therefore incubate the tubes and plate for several days before discarding them as non-viable.

3. Incubation

Incubate under the conditions specified for the strain in the delivery note or our on line catalogue (www.cect.org)

Before discarding sterilize all the remains of the original ampoule

Please note that some strains have a long lag phase and may need to be incubated up to two weeks or more

Important recommendation:

The strain should be plated again before use