INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

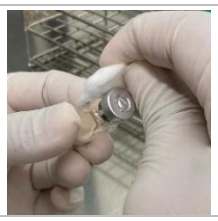
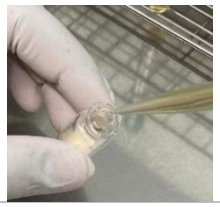
Important notes:

- Two different types of containers (vial and ampoule) are used. Please choose appropriate instruction.
- Aseptic technique must be applied throughout.
- All testing should be performed in a BSL2 level laboratory or in a biosafety cabinet class II.

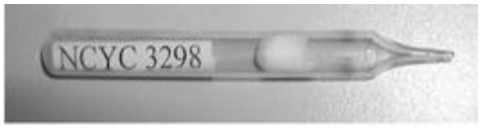
1. Vial

What is needed?
- Forceps for lid opening
- Tryptic soy broth (or other sterile microbiological diluents)
- Agar plates or broth suitable for bacterial species
- Autopipette with tips or Pasteur pipettes
- Inoculating loop

Upon receipt of samples ensure that the content is dry and protected from light. The test strains represent bacterial organisms in the form of freeze-dried vials. Check the sample numbers on the label on the vials.

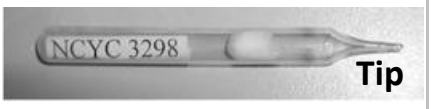
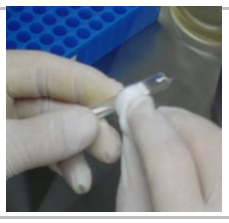
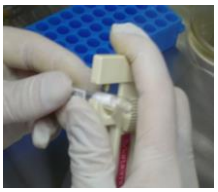
1. Perform testing as soon as possible. Keep samples in the dark at 2-8 °C until testing.	
2. Disinfect the lid of the vial with alcohol dampened gauze or cotton. Aseptically open the vial.	
3. Slowly add 1.0 mL tryptic soy broth (or other sterile microbiological diluents) using sterile Pasteur pipette and mix carefully to avoid creating aerosols until the pellet has dissolved.	
4. Leave for 10 minutes. Transfer the content to one or more suitable solid and/or liquid media.	
5. Autoclave or disinfect effectively the used Pasteur pipette and the remains of the original vial before discarding.	

2. Ampoule



What is needed?

- An ampoule cutter or a file
- Sterile Luria Bertani (LB) broth
- Agar plates (5 to 6 plates per one strain)
- Autopipette with tips or Pasteur pipettes
- Inoculating loop

1. Carefully take the ampoule out of the wrap. Note: To maintain the vacuum condition, **do not break the tip of the ampoule**. Otherwise, the air will enter the ampoule and the cotton wool plug will be pushed down and in contact with dried bacterial culture. If it happens, please simply remove the cotton plug with forceps. Note: The ampoule can be cut in the middle of below the cotton wool plug.	
2. Wipe the ampoule neck with 70% alcohol-dampened cotton wool.	
3. Make a deep score on the around the circumference of the ampoule near the middle of the plug using ampoule cutter or a file. Note: The ampoule should be cut in **the middle or below** the cotton wool plug.	
4. Wrap thick cotton wool around the ampoule and break at the marked area.	
5. Remove the pointed end of the ampoule and cotton into a biohazard container. Pipette 0.5 ml of sterile-appropriate broth into the dried cells, Luria Bertani broth for *Salmonella* and *E. coli* and Columbia broth into for *Campylobacter*. Mix gently and carefully to avoid creating aerosols	
6. For *Salmonella* and *E. coli*, transfer one drop of each strain onto one LB agar plate using or autopipette or pasture pipette. For *Campylobacter*, transfer one drop of each strain onto one mCCDA agar plate using or autopipette or pasture pipette. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.	
7. For *Salmonella* and *E. coli*, incubate the inoculated plates and the suspension tubes at 37^0C overnight and observe the bacterial growth. For *Campylobacter*, incubate the plates and the suspension tubes at 42^0C, 48 hours.