

## **INSTRUCTIONS FOR REVIVAL OF CULTURES SUPPLIED AS GELATINE DISCS**

To revive gelatine discs, follow the procedure described below. Use the media and cultivation conditions recommended in Delivery Slip - Attachment for cultivation. Most strains of microorganisms can be cultivated on a slant agar. To revive anaerobic bacteria, use cultivation in the high column of appropriate liquid culture media.

### **Aerobic bacteria and yeast (cultivation on slant agar):**

1. Remove the vial with gelatine discs from the refrigerator and leave at room temperature for 10 minutes to prevent undesirable condensation of water in the vial after opening.
2. Prepare slant agar tube. Condensed water should be present on the bottom of each slant agar tube; if not, please add a few drops of appropriate sterile broth medium or sterile distilled water.
3. Touch a disc with a flame, gently warm inoculating needle and transfer the stuck disc to the water condensed on the bottom of the slant agar tube.
4. Incubate at optimum cultivation temperature, 1-4 hours.
5. Tilt the tube and let the suspension run over the surface of the slant agar.
6. Incubate under optimal conditions, usually 24-48 hours.

### **Anaerobic bacteria (cultivation in liquid medium):**

1. Remove the vial with gelatine discs from the refrigerator and leave at room temperature for 10 minutes to prevent undesirable condensation of water in the vial after opening.
2. Touch a disc with a flame, gently warm inoculating needle and transfer the stuck disc to the tube with column of appropriate liquid culture media at least 10 cm high.
3. Incubate under optimal conditions, usually 24-48 hours.

### **Filamentous fungi (cultivation on a Petri dish):**

1. Remove the vial with gelatine discs from the refrigerator and leave at room temperature for 10 minutes to prevent undesirable condensation of water in the vial after opening.
2. Touch a disc with a flame, gently warm inoculating needle and transfer the stuck disc to the centre of the Petri dish, add about 5 drops of sterile distilled water to gelatine disc.
3. Leave it at room temperature for 30 minutes.
4. Spread the spore suspension over the surface of the Petri dish by sterile inoculating loop.
5. Incubate under optimal conditions, usually 3-5 days.

*For the preparation of other subcultures use a new disc.*