

REVIVAL OF CULTURES

Care should be taken when opening ampoules of freeze-dried cultures as the contents are in a vacuum.

Make a file cut on the ampoule at the mid-point of the cotton wool plug and crack the glass by applying a red hot wire or glass rod to the file cut. Allow air to enter slowly before gently removing the pointed part. If the ampoule proves difficult to crack, use the ampoule snappers provided (place one at either side of the file cut) and physically snap the ampoule using thick wadding or gloves to protect the operator. Discard the upper part of the ampoule and the cotton plug into a disinfectant solution.

After flaming the open end of the ampoule, up to 0.5 ml of a suitable liquid medium should be added to the ampoule and the contents mixed, avoiding frothing. The suspension should be sub-cultured into suitable solid and liquid media. The numbered filter paper strip should be transferred on the tip of a Pasteur pipette or with a loop to the surface of the solid medium with the number upwards. Ready recognition of the culture is thus ensured and confusion is avoided if several ampoules are opened at the same time. If a liquid medium only is used for resuscitation, then the paper strip should be placed into an inoculated tube of medium. It is advisable to prepare more than one subculture from the ampoule contents as a precaution against accidents. Sub-cultures should be incubated at the optimum temperature for the organism under appropriate gaseous conditions.

Resuscitated freeze-dried cultures tend to exhibit a lengthened lag period, but if after prolonged incubation the culture appears to be non-viable, the Collections should be informed. The number and date on the paper strip should be quoted, together with details of the growth medium and its pH, the incubation temperature and time, and any gas mixture employed.

Ampoules not opened soon after receipt should be stored in a cool, dark place (e.g. a refrigerator). They should not be stored exposed to light, particularly direct sunlight. N.B. Organisms should be sub-cultured at least twice before they can be optimally used in experiments.



The Catalogue number of the culture is read from the rounded end of the ampoule: thus the above number is 9086 not 9806. The date the culture was freeze-dried is stamped on the reverse side of the paper slip.

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