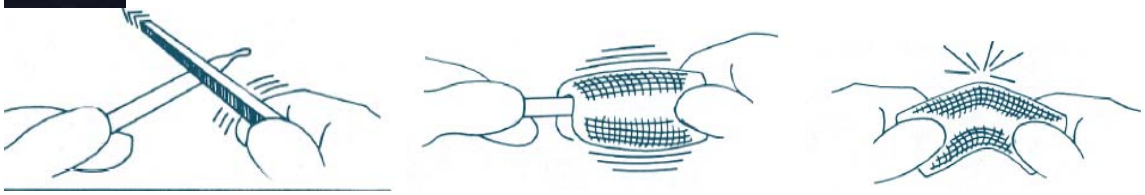
Techniques for Reconstituting Freeze-dried Ampoules (Lyophils)

See below for detailed instructions. Wear gloves and work in biosafety cabinet. To open vial:
1. Score vial with glass cutter on one side of vial near midpoint as shown on dotted line.
2. Wrap vial with gauze or paper towel moistened with 70% alcohol. Hold vial in both hands with fingers positioned near score.
3. Snap ampoule at score mark by pushing up with thumbs.



Drawings from ATCC website.

Have ready:

- ✓ Sterile water [or substitute nutrient broth for bacteria and some fungi]
- ✓ Plate(s) of a suitable medium for growth of the fungus (PDA, CER or other). A medium without antibacterial antibiotics is recommended so that contaminating bacteria may be detected, if present.
- ✓ Pasteur pipette and bulb
- ✓ Glass scorer or file
- ✓ Sterile paper towel or gauze or small pieces of sterile cloth
- ✓ Surgical gloves

Procedure

- Open ampoule within a laminar flow or biological safety cabinet. Wear gloves.
- Record UAMH number on bottom of agar plate.
- Wipe exterior of ampoule carefully with gauze, Kimwipe, Kleenex or sterile cloth moistened with 70% alcohol or soak vial in disinfectant for 30 sec. **CAUTION:** Inked numbers may be washed off vial.
- Score the vial with glass cutter on one side. Score should be near the centre and slightly above the freeze-dried material (see dotted line above).
- Wrap sterile paper towel, gauze or cloth dampened with 70% alcohol around the vial and snap open by pressing on each side of the score mark with thumbs. Avoid overwetting and aspiration of alcohol into vial. Discard top of vial.
- Using pasteur pipette, flood lyophilized contents in bottom of vial with sterile water. Suspend the material *thoroughly* by drawing a portion up into the pipette several times and by using the tip of the pipette to scrape bits from the side of the vial.
- Use pipette to transfer **all** suspended material and larger particles to agar plate.
- Leave plates lid side up for 24-48 hours until liquid is absorbed into the medium, then invert (lid down).
- Incubate at recommended temperature (usually 25 to-30 C).
- Monitor plate for growth. Growth should occur within 2 to 3 weeks but hold 4-5 weeks before discarding plates.

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