TECHNIQUES FOR RECONSTITUTING PRESERVED STOCKS

May 5, 2005

Reconstituting Freeze-Dries (Lyophilized Ampoules)

Have ready:
- ✓ sterile water or nutrient broth
- ✓ plate(s) of a suitable medium for growth of the fungus (PDA, CER or other)
- ✓ pasteur pipette and bulb
- ✓ glass scorer or file
- ✓ sterile paper towel or small pieces of sterile cloth
- ✓ surgical gloves if the organism is known to be infectious

Procedure
- Open ampoules within a laminar flow or biological safety cabinet if available.
- Record UAMH number on agar plate.
- Soak vial in disinfectant for 30 sec. or wipe exterior of ampoule carefully with clean Kimwipe, Kleenex or sterile cloth moistened with 70% alcohol**. CAUTION** Inked numbers may be washed off vial.
- Open the vial near the centre and slightly above the dried material by first scoring the glass with a sharp file or glass cutter.
- Wrap sterile paper towel or cloth dampened with 70% alcohol around the vial and snap open by pressing on each side of the score mark with thumbs. Surgical gloves may be worn to open the vial.
- Using pasteur pipette, add sterile water to the bottom part of the vial containing the lyophilized contents
- 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.
- 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.
- Growth should occur within 2 to 3 weeks but hold 4-5 weeks before discarding plates.
- Incubate at 25-30 C unless advised to use higher or lower temperatures of incubation.

Reconstituting Liquid Nitrogen Straws

Have ready:
- ✓ plate(s) of a suitable medium for growth of the fungus (PDA, CER or other)
- ✓ Pasteur pipette and bulb
- ✓ sterile wooden applicator sticks
- ✓ scissors
- ✓ Kimwipe or Kleenex
- ✓ sterile plastic petri dish
- ✓ surgical gloves if the organism is known to be infectious

Procedure
- Thaw for 5 minutes by warming straw in beaker of clean water warmed to 35 C.
- Open straws within a laminar flow or biological safety cabinet if available.
- Record UAMH number on plate**.**
- Using a Kimwipe or Kleenex, **wipe the exterior of the straw** with 70% alcohol. Caution: numbers may wipe off.

For organisms cryopreserved as suspensions:
- With sterile scissors, **snip off one end** of the straw
- Pipette the suspended contents onto the agar surface of the labeled petri plate.

For organisms cryopreserved as plugs:
- Hold straw over open plastic petri dish.
- Snip off both ends of the straw.
- Using a sterile applicator stick, push the plugs out onto labelled plate of agar medium (PDA or other).
- Spread the plugs out and away from each other and the residual cryoprotectant.

- Leave plates lid side up for 24-48 hours until liquid is absorbed into the medium, then invert.
- Incubate at 25-30 C unless advised to use higher or lower temperatures of incubation.
- Growth should occur within 2 to 3 weeks but hold 4-5 weeks before discarding plates.

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