INTRODUCTION

Lyophilization, or freeze drying, can be used to prepare plasma that will remain stable over prolonged periods of time. This is useful, for example, for preparation of lyophilized FVIII-deficient plasma, which is normally stable for at least two and up to five years when stored at -20°C or lower, and which is stable enough to survive short periods (up to seven days) at temperatures of 20°C–25°C.

SUITABLE PLASMA

Venous blood mixed with 0.105–0.109M sodium citrate in the proportion 9 parts blood, 1 part anticoagulant. Centrifuge at 1700 g for 10 minutes, pool as appropriate, and store at -55°C pending viral test results. Confirm negative results for anti-HIV 1 and 2, anti-HCV, and Hep B SAg.

MATERIALS

- 2 ml clear neutral glass vials with internally siliconized 13 mm neck
- 13 mm freeze-dry stopper, grey
- 13 mm fully tear-off seals

(All available from Diagnostic Reagents, Thame, Oxfordshire, U.K.)
- Freeze-dryer unit (Supermodulyo and stoppering shelf unit available from Life Sciences International, Unit 5, Ringway Centre, Edison Road, Basingstoke, Hampshire, RG21 6YH, U.K.)
- N-2-Hydroxyethylpiperazine-N’-2-ethanesulphonic acid (HEPES; BDH Laboratory Suppliers, Poole, BD15 1TD, U.K.)

METHOD

1. Thaw plasma rapidly at 37°C.
2. Mix well.
3. Add 0.8 g HEPES per 100 ml plasma.
4 Mix and allow HEPES to dissolve (about 15 to 20 minutes).

5 Fill stainless steel lyophilization trays with empty vials.

6 Dispense exact 0.5 ml aliquots in each vial.

7 Place rubber bung in each vial to depth of narrow ridge on bung. Ensure air access out of vial.

8 Freeze at -70°C for a minimum of three hours.

9 Turn on freeze-dryer unit. Activate fridge unit.

10 Place trays in shelving unit (up to eight).

11 Place shelving unit in clear plastic chamber over air exit port on top of freeze-dryer.

12 Activate pump. Ensure that the above two steps and this step are completed within three to four minutes to prevent plasma thaw commencing. If partial thaw occurs, material will froth and freeze dry poorly, and it must be discarded.

13 Confirm that vacuum is developing by movement in pressure gauge (visible movement within a few minutes) and by immobility of plasma chamber under light lateral manual pressure.

14 Leave under vacuum for five days.

15 Seal vials under vacuum by screwing down handles on shelving unit.

16 Allow air access through entry port.

17 Defrost and dry freeze-dryer.

18 Store lyophilized plasmas at -20°C, and cap as soon as possible.

19 To confirm even lyophilization of plasma, test four to six vials selected from different locations within the steel trays. Determine PT and APTT, which should not vary by more than 6%–8%.