Preparation and Calibration of Pooled Normal Plasma (PNP)

Figure 7.1. Pooled normal plasma collection

<table>
<thead>
<tr>
<th>Donors</th>
<th>Minimum 20 normal healthy individuals not taking medications that interfere with clotting factors and coagulation reaction. It is acceptable to include women taking oral contraceptives. An approximately equal number of males and females is desirable. The age range should be 20 to 50 years.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulant</td>
<td>0.109M (3.2%) trisodium citrate dihydrate buffered with N-2-hydroxyethylpiperazine. N-2-ethanesulphonic acid (HEPES) at 5 g per 100 ml trisodium citrate.</td>
</tr>
<tr>
<td>Collection</td>
<td>Donors are bled between 9:00 a.m. and 11:00 a.m. using 60 ml plastic disposable syringes and 21 g butterfly needles.</td>
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</tbody>
</table>

METHOD FOR PREPARATION OF PNP

1 Collect 54 ml blood and mix with 6 ml anticoagulant in plastic containers.
2 Store sample on melting ice during preparation of pool.
3 Centrifuge at 4°C for 15 minutes at 2500 g.
4 Pool plasma in plastic non-contact container.
5 Aliquot in 1.5 ml plastic vials in 0.5 ml aliquots.
6 Snap freeze on dry ice/solid CO₂ if available. Alternatively, place immediately on an open shelf at -70°C.
7 Complete above procedure within four hours.
8 Stable at -70°C for > six months.
A pooled normal plasma (PNP) prepared in this way will have levels of factors II, V, VII, IX, X, XI, XII, HMWK, and prekallikrien of around 1 U/ml or 100 U/dl, although the levels of FVIII and von Willebrand factor (VWF) vary widely in different pools of PNP. Such a local PNP should be calibrated in International Units (IU), since international standards are now available for all the above-mentioned clotting factors, with the exception of FXII. The pool can be used uncalibrated with an assumed potency of 100 U/dl or 1 U/ml for FXII. To calibrate in IU, it is necessary either to obtain calibrated WHO reference preparations (which are held at the National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts, U.K.) or to purchase a suitable commercial reference plasma that has been calibrated in IU by the manufacturer. Consideration should be given to replacing such a plasma pool every 12 to 18 months, unless there is evidence from internal quality control results that stability has been maintained.

METHOD FOR CALIBRATION OF LOCAL PNP

1. Obtain calibrated standard e.g. WHO International Standard (IS) (minimum two vials).

2. On two different days, use one vial of IS and four aliquots of local PNP.

3. On day one test IS, local, local, local, local, IS, and repeat this using fresh dilutions of each plasma.

4. On day two test local, local, IS, IS, local, local, and repeat this using fresh dilutions of each plasma.

5. Calculate potency of each aliquot of local standard against average of results with the two IS.

The mean result of 4 aliquots × 2 dilutions × 2 days (n = 16) is assigned to the local standard as its potency.