



Copenhagen, November 2003

RECONSTITUTION OF THE LYOPHILIZED HUMAN SERUM STANDARD FOR MANNAN-BINDING LECTIN (MBL)

The standard was produced from pooled donor serum. Individual donations were tested negative for HbsAg and antibodies against HIV 1 and 2 and HCV.

Blood from 30 healthy donors was collected in flasks without anticoagulant and allowed to clot. Serum was collected after centrifugation and pooled in a 10-liter flask. After mixing, 1-ml aliquots of the serum was pipetted into 2-ml vials. Each vial was assigned an MBL content of 1000 arbitrary units (AU). The material was lyophilized and the vials closed under vacuum. The standard is kept at -20°C and has been shown to be stable for several years under these conditions.

To obtain reliable and comparable results it is important to determine the exact concentration of MBL in ng/ml after reconstitution of the lyophilized MBL with sterile-filtered deionized water.

Please read and carefully follow the 7 steps set out below:

STEP

ACTION

- | STEP | ACTION |
|-------------|--|
| 1 | Take 1 vial from storage at -20°C and leave at room temperature for 1 hour |
| 2 | Remove cap from vial |
| 3 | Add 1 ml sterile-filtered deionized water to reconstitute the lyophilized material in the vial. This will result in slightly more than 1 ml of solution. |
| 4 | Carefully wash the solution into a 10-ml volumetric flask or 10-ml measuring cylinder with sterile-filtered deionized water and make up the volume to 10 ml with water. This will produce 10 ml of solution containing 100 AU of MBL per ml. |
| 5 | Perform a double determination of MBL in ng/ml using your in-house method |
| 6 | Calculate the amount of MBL in ng/AU or AU/ng |
| 7 | Aliquot portions of 500 µl into appropriate vials and store at -20°C |