Obtaining Human Peripheral Blood Cells

Peripheral blood is the chief source of mononuclear cells (lymphocytes and monocytes) for immunologic studies of humans. Such blood can be obtained by simple venipuncture when relatively small amounts of blood (10 to 100 ml) are necessary or by lymphapheresis when large amounts (300 to 5000 ml) are necessary. Cells collected by these procedures can be further separated by techniques described in Chapter 7.

CAUTION: When working with human blood, cells, or infectious agents, biosafety practices should be followed (see Chapter 7 introduction).

NOTE: All solutions and equipment coming into contact with cells must be sterile, and proper sterile technique must be used accordingly.

BLOOD COLLECTION BY VENIPUNCTURE

Materials

- Heparin, preservative-free (optional)
- Vacutainer needles (20- to 21-G) and needle holders or equivalent (e.g., Scientific Products), or syringe with 17- to 21-G and 23-G needles
- Anticoagulant (e.g., heparin-containing) blood collection tubes (e.g., Scientific Products)
- Sterile gauze pads, dry and soaked in iodine and 70% isopropanol
- Tourniquet and bandage

1. Sterilize the skin area where the needle is to be inserted by vigorously washing the area with an iodine-soaked gauze pad followed by an alcohol-soaked gauze pad. The antecubital fossa is the preferred site because of the size of the vessels and because veins in this site tend to be fixed in one place.

2. Assemble needle, needle holder, and evacuated anticoagulant tube according to manufacturer’s instructions.

   A pediatric scalp vein butterfly needle may be used to facilitate collection of blood. It is connected to the evacuated blood collection tube or heparin-containing syringe via a flexible plastic tube, allowing movement of the tube or syringe during the blood-drawing procedure. Blood can be collected into multiple tubes or syringes as for the traditional Vacutainer setup.

   Alternatively, draw preservative-free heparin into a sterile syringe through a 21- or 23-G needle; enough heparin should be drawn so that the final heparin concentration (after total blood sample is collected) is ~5 U/ml. Remove needle and replace with an appropriately sized needle for obtaining blood.

3. Place a tourniquet around the upper arm to partially occlude venous return; insert needle (bevel up) into the vein, and slowly withdraw blood.

   From an adult donor, 60 ml can be safely withdrawn at one time. Total collection should not exceed 400 ml over a 4-week period.

4. Following blood collection, remove tourniquet, then remove the needle with one hand and with the other hand press a sterile gauze pad over the needle puncture site. Hold the gauze pad in this position for 3 to 5 min or until the puncture is closed. Place a sterile bandage over the venipuncture site.
**BLOOD COLLECTION BY LYMPHAPHERESIS**

Obtaining blood by lymphapheresis (also known as leukapheresis) requires access to a blood bank facility where lymphapheresis is routinely performed. Using a basic blood-cell separator (e.g., Haemonetics V50), a relatively small amount of blood (~30 ml) is drawn and separated by centrifugation into leukocytes and erythrocytes; the former are collected for use and the latter are returned to the donor (using the same venous access site used for drawing blood). The process is then repeated until the desired number of leukocytes are obtained. The separator can be set to collect only lymphocytes and most macrophages, and to exclude granulocytes; alternatively, all white cell elements can be collected. A two-unit lymphapheresis (~1 liter) using this separator should yield $1 \times 10^9$ cells from individuals with normal white cell counts. The procedure takes 1 to 2 hr per donor.

Using a more complex separator, such as a Fenwal CS-3000 (Baxter Healthcare), leukocytes and erythrocytes are separated and collected/returned in a continuous fashion. For this reason, the separator utilizes one venous access site as an outflow track and another access site as an inflow track. Cells in up to 5 liters of blood (as many as $4 \times 10^9$ mononuclear cells) can be obtained by this procedure, which also takes ~ 1.5 hr per donor.

Mononuclear cells obtained with the use of cell separators should contain most of the macrophages present in whole blood and—with the proper settings—few if any granulocytes. However, the machine can be set so that granulocytes can also be collected. The cells obtained by lymphapheresis may contain excess platelets. These can be removed by subjecting the cells to Ficoll-Hypaque gradient centrifugation (**UNIT 7.1**).

The anticoagulant used in cell separator devices is acid/citrate/dextrose. The cells should thus be washed prior to storage or use. This is accomplished by resuspending the cells in 120 ml complete RPMI-10 (**APPENDIX 2**) and proceeding with wash procedure as described in **UNIT 7.1**.

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