Introduction

Blood plasma is the liquid component of blood, in which the blood cells are suspended. It makes up about 60% of total blood volume. It is composed of mostly water (90% by volume), and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide (plasma being the main medium for excretory product transportation). Plasma is the supernatant fluid obtained when anti-coagulated blood has been centrifuged. The blood is mixed with an appropriate amount of anticoagulant like heparin, oxalate or ethylenediaminetetraacetic acid (EDTA). This preparation should be mixed immediately and thoroughly to avoid clotting.

Blood serum is blood plasma without fibrinogen or the other clotting factors. Serum is clearer than plasma because of fewer proteins. Proteins are sometimes considered as interfering substances in some tests as they react with the reagent and thereby yield inaccurate results. Serum is the preferred specimen in clinical testing as the interference that may be caused by a plasma specimen because of the presence of an anticoagulant, is eliminated.

Process samples as soon as possible. If storage is necessary prior to processing, store the blood at room temperature, shielded from light, and on a rocker. DO NOT refrigerate the cells.

Blood Plasma Preparation

Materials and Equipment

- Human blood sample.
- Vacutainer tubes containing anticoagulant (e.g. BD Vacutainer plastic EDTA tube, 10 ml, lavender top (#366643))
- Serological pipettes of appropriate volumes (sterile)
- Centrifuge tubes
- Cryovials
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure

1. **Draw blood into vacutainer tube(s) containing ~1.8 mg K$_2$EDTA per ml blood (may vary depending on manufacturer).** Be sure to draw the full volume to ensure the correct blood-to-anticoagulant ratio.
2. **Invert vacutainer tubes carefully 10 times to mix blood and anticoagulant and store at room temperature until centrifugation.**
3. **Samples should undergo centrifugation immediately.** This should be carried out for a minimum of 10 minutes at 1000-2000 RCF (generally 1300 RCF) at room temperature (refer to speeds and times recommended by manufacturer). **Do not use brake to stop centrifuge.**
4. **This will give three layers: (from top to bottom) plasma, leucocytes (buffy coat), erythrocytes.**
5. Carefully aspirate the supernatant (plasma) at room temperature and pool in a centrifuge tube. Take care not to disrupt the cell layer or transfer any cells.

6. Inspect plasma for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

7. Aliquot plasma into cryovials and store at –80 °C. Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.

Blood Serum Preparation

Materials and Equipment

- Human blood sample
- Vacutainer tubes (containing either no additive or a clot activator)
  - Clot activator and silica gel:
    - e.g. BD Vacutainer Plus plastic serum tube, 10 ml, red top (#367820)
    - e.g. BD Vacutainer Plus plastic serum tube (transport tube), 10 ml, mottled red/grey top (#367985)
  - No additive:
    - e.g. BD Vacutainer Plus tube with clear BD Hemogard closure, 3 ml, clear top (#366703)
- Serological pipettes of appropriate volumes (sterile)
- Centrifuge tubes
- Cryovials
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure

1. Draw whole blood into vacutainer tube(s) containing no anticoagulant. Draw approximately 2 ½ times the volume needed for use e.g. 10 ml blood for 4 ml serum.

2. Incubate in an upright position at room temperature for 30-45 min (no longer than 60 min) to allow clotting. If using a clot-activator tube, invert carefully 5-6 times to mix clot activator and blood before incubation.

3. Centrifuge for 15 min at manufacturer’s recommended speed (usually 1000-2000 RCF). Do not use brake to stop centrifuge.

4. Carefully aspirate the supernatant (serum) at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use a clean pipette for each tube.

5. Inspect serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.

6. Aliquot into cryovials and store at –80 °C. Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.