Serum Isolation Using Serum-Separating Tubes

Serum is the liquid component of blood that contains water, electrolytes, and proteins including antibodies and hormones, but lacks clotting factors. Serum is recovered from whole blood after allowing the blood to clot.

Serum-separating tubes (SST) contain a gel and clot activator. After centrifugation, the gel forms a barrier between serum and clotting factors such as fibrin. It also prevents exchange of substances between blood cells and serum.

Isolated serum is used for numerous applications including:

- Blood typing
- Diagnostic tests for infectious diseases
- Measurement of analytes such as biomarkers, drugs, hormones
- Lipid panel testing
- Liver panel testing
- Blood glucose measurement

Here, we describe a step-by-step protocol for isolation and collection of serum from SST blood tubes.

Reagents and Materials

- Venous blood collected in Red Tiger-Top BD Vacutainer™ SST tubes
- Pipettes and pipette tips

Equipment

- Venous blood collected in Red Tiger-Top BD Vacutainer™ SST tubes
- Pipettes and pipette tips

Procedure

1. Measure and record the estimated total blood volume collected. Gently invert the SST tubes about 5 times to mix the clot activator and blood.
2. Allow the blood to clot at room temperature (18 °C to 25 °C) for 30–60 minutes.
3. Centrifuge the tubes at 800–1,000 x g at room temperature (18 °C to 25 °C) for 10 minutes with the BRAKE OFF to avoid disrupting the density gradient during deceleration.
4. The following layers should be visible after centrifugation (from top to bottom, **Figure 1**):
    - **Yellowish serum layer (supernatant)**
    - Silica gel barrier layer
    - Deep red layer of erythrocytes

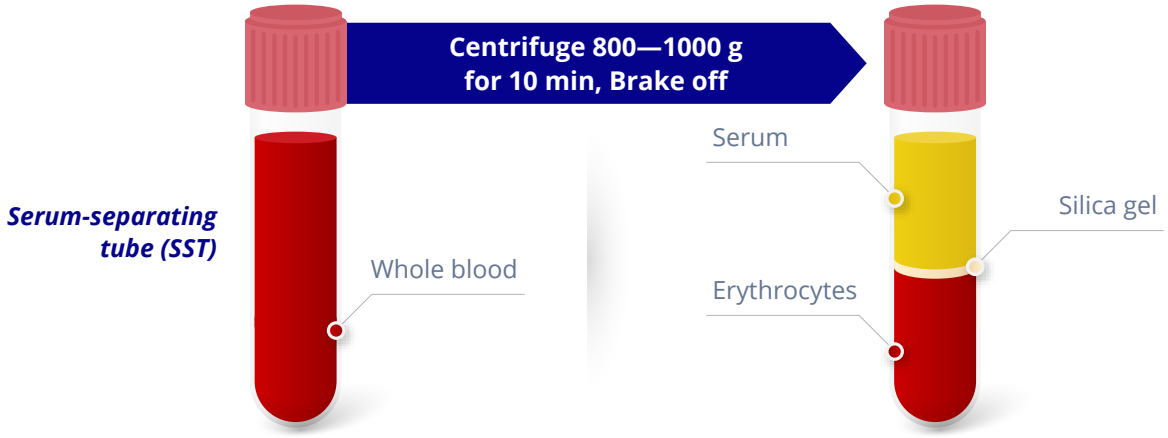

FIGURE 1. Serum isolation using SST tubes.

5. Using a pipette, aliquot 1 mL of serum into 2 mL cryovials for storage or transportation. Samples should be maintained on ice at 2 °C to 8 °C during handling.

   Note: Do not disturb the silica gel layer while collecting serum.

6. Store the cryovials containing serum at -80 °C.

   Note: Avoid multiple freeze-thaw cycles of serum aliquots as temperature variations can negatively impact sample quality.

Troubleshooting

Problem	Potential Solutions
Lack of distinct layers after centrifugation	▪ Perform centrifugation with brakes off. Deceleration disrupts the formation of distinct layers. ▪ Weigh tubes to ensure rotors are appropriately balanced. This avoids excessive shaking during centrifugation.
Pink or reddish tinge to serum	▪ *In vivo* or *in vitro* hemolysis of red blood cells may impart a pink or reddish tinge to serum. In vivo hemolysis may be attributed to diseases such as hemolytic anemia. Further investigation of the patient's clinical history may be warranted. ▪ *In vitro* hemolysis may occur during sample collection, handling, and storage. Ensure samples are maintained at 2 °C to 8 °C during handling and stored at -20 °C or below.