Specimen Collection and Processing Guidelines for Buffy Coat and Plasma

If protocol-specific collection instructions for peripheral blood and processing instructions for buffy coat and plasma are not provided in the protocol or through a linked resource (usually in Section 15 in SWOG-led protocols), then follow the instructions outlined below.

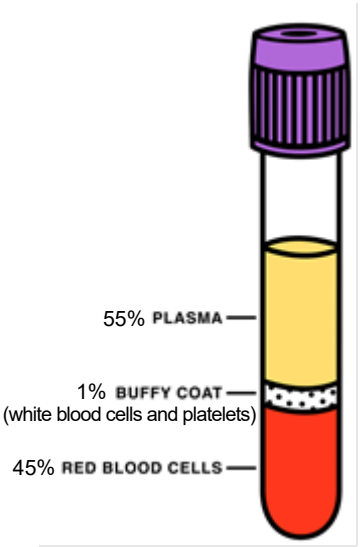
Plasma is processed from blood collected with anticoagulant (e.g. EDTA, sodium heparin, etc.). Inverting the tube immediately after collection is essential to ensure blood does not clot. Plasma and buffy coat are processed by centrifuging and removing the yellowish-clear layer (plasma) and/or the very thin white or gray-ish layer (buffy coat) – see figure. Note: after processing, plasma looks very similar to serum. If a protocol includes both plasma and serum specimens, it's *imperative* that each tube is labeled with the specimen type (e.g., plasma or serum).

Collecting Peripheral Blood

1. Use the protocol-specified Vacutainer tube type.
   - If the recommended size of vacutainer tube specified in the clinical trial protocol is not available, then other sized tubes may be used to collect the total volume of blood (e.g., if 10 mL of blood is requested, then two (2) 5-mL tubes may be used).
   - Pre-label vacutainer tube(s) according to specimen labeling requirements.
2. Draw blood from the participant into the vacutainer tube(s). The amount of blood required will vary per protocol; refer to section 15 for the collection volume.
3. Immediately after collection, gently invert the tube 5-10 times to thoroughly mix the blood with the anticoagulant and prevent clotting.
4. Blood must be processed within 2 hours after venipuncture unless otherwise noted in the protocol. Document on the specimen shipping form if the blood was not processed within 2 hours following venipuncture.

Plasma Processing

1. Centrifuge the vacutainer tube(s) at 1200 x g for 10 minutes at room temperature.
2. Pre-label cryovials according to specimen labeling requirements.
   For best results: Label specimens prior to freezing. Use laboratory marking pens or a cryogenic marker to label frozen biofluids and use labels that are designed to adhere to frozen surfaces.
3. Using a clean disposable pipette, remove the plasma (yellow-clear liquid above the buffy coat and red blood cell layers). See Figure. No cells or debris should be present in the plasma.
4. Dispense 1 mL aliquots of plasma into the pre-labeled 2 mL-capacity cryovials and cap the tubes securely. If the aliquot volume is not specified in the protocol, use as many cryovials as needed to evenly dispense plasma into 1 mL aliquots.
   - The number of vials needed will vary based on the volume of plasma obtained but can be estimated as roughly half of the blood volume collected.
5. Immediately freeze plasma vials in an upright position, buried in dry ice or in a -70°C to -80°C freezer until ready to ship.
6. If buffy coat is also required, follow instructions below to remove buffy layer. If buffy coat is not requested, then discard remnant cells.



Buffy Coat Processing

1. Centrifuge vacutainer tube(s) at 1200 x g for 10 minutes at room temperature. *Note: if processing plasma and buffy coat, only one centrifugation is needed.*
2. Pre-label cryovials according to specimen labeling requirements.
   For best results: Label specimens prior to freezing. Use laboratory marking pens or a cryogenic marker to label frozen biofluids and use labels that are designed to adhere to frozen surfaces.
3. Using a clean pipette, slowly remove the buffy coat (the thin, cloudy pin or gray-white layer located in between the red blood cells and the plasma; refer to figure below). Avoid aspirating the red blood cells while collecting the buffy coat.
4. Split the buffy coat equally into two 2 mL cryovials.
5. Immediately freeze vials in an upright position, buried in dry ice or in a -70°C to -80°C freezer until ready to ship.

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