Blood Sample Collection

Preparing to submit a sample

For tubes commonly collected for the clinical laboratory testing, the tube order recommended by the National Committee for Clinical Laboratory Standards (NCCLS) is blue (citrate), red (serum/clot), green (heparin), then lavender (EDTA).

- Fasted samples (monogastric animals) are recommended to avoid lipemia.
- Choose the largest vein and largest needle that can safely be used.
- Use a 21-22G needle for small animals and an 18-20G needle for large animals.
- Never use a needle smaller than 23G.
- Minimize trauma to the vein (e.g., do not wipe aggressively with alcohol, do not hold vein off for greater than 10 seconds prior to blood draw).
- Use minimal suction when drawing blood into syringe.
- Blood can be collected into a 3-5mL syringe and transferred into a vacuum tube by puncturing the rubber stopper of the vacuum tube with the needle, letting the vacuum action of the tube draw the sample out of the syringe and into the tube.
  - » **Do not apply extra pressure to the syringe plunger.**
  - » **If the vacuum fills, you may gently depress the syringe plunger to fill blood tubes to the indicator line.**
  - » **Do not under or overfill blood collection tubes.**
- Blood can also be collected using a butterfly needle with a short catheter line when clinically indicated.
- Blood should not be collected through an established IV catheter.
- All tubes **must** be labeled with the patient's first and last name.

Whole blood – best practices

- Whole blood samples may be collected in EDTA, sodium citrate, or lithium heparin-containing anticoagulant tubes. EDTA whole blood is most commonly used for CBCs. A CBC can be performed on sodium citrate, or lithium heparin whole blood; however analyzers and reference intervals are not typically validated for these sample types.
- Select the correct tube size for the sample volume.
  - » **All tubes should be filled to at least the minimum sample volume fill line indicated on the tube** (please note that fill lines on some tubes may not be conspicuous).
  - » **UNDERFILLING EDTA TUBES CAN CAUSE THE FOLLOWING ARTEFACTS: decreased MCV, increased MCHC, and decreased HCT. Filling an EDTA tube with 50% or less of the recommended fill volume can decrease the HCT by up to 25%.**
  - » EXAMPLE: 2 mL EDTA tube appropriately filled HCT = 45%, same original sample but only 0.25 mL put into 2 mL EDTA tube yields HCT = 34%.
- **Sodium citrate tubes should be filled exactly to the fill line to ensure a 1:9 ratio of anticoagulant to blood. UNDERFILLING SODIUM CITRATE TUBES LEADS TO:** A relative excess of sodium citrate in the sample. When the sample is then utilized for coagulation assays, this excess sodium citrate can slow the reactions, **resulting in artefactually prolonged clotting times.** Similarly, overfilling sodium citrate tubes can artefactually shorten clotting times.
- Microtainer anticoagulant tubes are available if only a small (<1.0 ml) volume of blood can be obtained.
- Once filled, tubes should be immediately, gently inverted manually 10 times to ensure proper mixing of anticoagulant and blood.

  - » Special care is needed inverting microtainer tubes to ensure that blood is fully mixing.
    - Rolling microtainers between your hands can better mix blood and anticoagulant in these tubes.
- Never shake blood tubes.
- Using a tube rocker for initial mixing of blood with anticoagulant is not sufficient as this motion will not fully mix the sample.
- Whole blood samples that will not be evaluated immediately should be refrigerated and protected from light but never frozen.
- Two unstained blood smears should be submitted with any EDTA blood for CBC.
  - » Blood smears should be air-dried. A hair dryer on the "cool" setting can be used to accelerate the drying process.
  - » Blood smears should be kept at room temperature and away from moisture and formalin fumes. Do NOT refrigerate blood smears.
- For shipping, whole blood samples should be packed with a cold pack (wrapped in paper towels/packaging paper), but never frozen or placed directly in contact with an ice pack in the shipping container.

Serum – best practices

- Collected into RTT (no additive, clot activator, or serum separator), "tiger top" tubes, gold top tubes.
  - » Serum should be separated from the cellular component of blood within 1 hr of collection.
  - » Blood collected in these tubes should be allowed to clot for 15-30 minutes and then centrifuged to separate cellular components from serum.
    - Longer times may be needed for large animal species.
    - Longer times will also be necessary if no clot activator is present in the tube.
- Once sample is clotted, centrifuge to separate serum from RBCs.
  - » If using a tube with serum separator gel, check the spun sample to ensure centrifugation has fully separated RBCs from serum within the tube. If not using a serum separator gel, the serum should be removed using a disposable plastic pipette and transferred to a sterile, no-additive (white top or no-additive red top) tube.
- Store serum in refrigerator until packaging/shipping/courier pick-up.
- Use a disposable plastic pipette to remove serum and transfer serum to a sterile, no additive tube (white top tube, no-additive RTT).
- **Be Sure to Label this Sample as 'SERUM' on the Tube.**
  - » Serum, plasma, and often urine cannot be differentiated visibly.
- **Please Note:** Visible hemolysis (pink to red serum) or lipemia (cloudy to milky white serum) may interfere with many assays. Some samples may be rejected or subject to erroneous results. Resampling may be necessary.
- For shipping serum that is intended for routine testing, ship packed with a cold pack (wrapped in paper towels/packaging paper). Specialized testing may require serum to be shipped frozen on ice or possibly frozen on dry ice.

Plasma – best practices

- Blood for plasma may be collected in EDTA, sodium citrate, or heparin-containing anticoagulant tubes. The type of plasma required will depend on the type of testing desired.
  - » **Immediately after collection and proper mixing with anticoagulant, the anticoagulated whole blood should be centrifuged to separate the plasma from RBCs.**
  - » **Plasma should be removed immediately and transferred to a sterile, no additive tube (white top tube, no-additive RTT) using a plastic pipette.**
    - **Please Note:** For sodium citrate samples intended for coagulation assays (e.g., PT, aPTT), plasma should be removed from RBCs immediately after sample collection. A delay in harvesting plasma may affect result accuracy.
    - Plastic or glass tubes are both acceptable for sodium citrate plasm samples intended for coagulation assays.
- **Be Sure to Label the Tube as Containing EDTA/Citrate/Heparin Plasma**
  - » Lavender top tube - EDTA PLASMA
  - » Blue top tube - CITRATE PLASMA
  - » Green top tube - HEPARIN PLASMA
  - » Serum, plasma and often urine cannot be differentiated visibly.
- **Please Note:** Visible hemolysis (pink to red plasma) or lipemia (cloudy to milky white plasma) causes interference with many assays. This may affect sample accuracy. In some cases, samples may be rejected. Resampling may be necessary.
- Plasma is typically used for specialized testing. Consult the shipping requirements for your desired test. Depending on the specific test shipping requirements, plasma samples may be packed with a cold pack (wrapped in paper towels/packaging paper), frozen on cold packs or possibly frozen on dry ice.

Urine collection – best practices

- Sample methods:
  - » Urinalysis may be performed on urine collected via all methods, but some sample collection methods limit culture options.
  - » Cystocentesis samples are the ideal sample for urine culture, as this method avoids lower genitourinary tract contamination.
  - » Transurethral catheter samples can be used for culture when collected aseptically, but can be contaminated with lower genitourinary tract bacteria if technique is flawed.
  - » Voided samples are ideally collected midstream and can be used for urinalysis, but are not ideal for culture due to the high degree of lower genitourinary tract contamination.
  - » Voided samples collected from floors or counter tops are unacceptable for culture.
- A minimum of 5 mL of urine should be collected for urinalysis whenever possible. 1 mL of urine is the absolute minimum for a complete urinalysis.
  - » Lower volumes will be accepted; however, not all components of the urinalysis (chemical analysis, sediment examination, urine specific gravity, +/- cytology and additional testing) may be performed in low volume samples.
  - » Examination of a urine sediment may not be possible in low volume samples.
- A minimum of 1 mL of urine should be collected for urine culture.
- Urine should be collected into a sterile, single-use yellow top urine tube with no additive.
- Samples received in reusable containers (e.g., jars, previously used plastic containers, cups with lids) are unacceptable as they are subject to contamination and often leak during transit.
- Samples that have opened or leaked during transit can sometimes be salvaged for urinalysis, but are unacceptable for culture.
- If urine is collected into a syringe with needle, fill the urine tube by piercing the needle through the rubber stopper.
  - » **If a tube must be opened to fill, be certain the lid/ rubber stopper is closed tightly and securely to avoid in-transit leakage.**
- All tubes must be labeled with the patient's first and last name.
  - » **Label the tube URINE, as serum, plasma, and urine cannot always be visibly differentiated.**
- Refrigerate urine samples that will not be processed within 30 minutes.
- For shipping, urine samples should be packed with a cold pack (wrapped in paper towels/packaging paper) but never frozen or placed directly in contact with an ice pack.

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