

SAMPLE COLLECTION

A number of detailed guidelines describe the procedures for collection and processing of blood samples for tests of hemostasis (CLSI 2007, 2008a, 2008b).

When possible, venous blood should be collected from veins in the bend of the elbow, using a tourniquet to facilitate collection. The tourniquet should be applied just before sample collection. The needle should not be more than 21-gauge for adults, and the sample should be collected using a syringe and/or an evacuated collection system that allows rapid collection of the blood sample. The blood should be drawn gently into the syringe. For infants, a 22- or 23-gauge needle may be necessary.

Any sample that is not obtained quickly with an immediately successful venipuncture should be discarded because of possible activation of coagulation. The blood should not be passed back through the needle after collection into a syringe. The needle should be removed before passing the blood from the syringe into the container with anticoagulant. There should be no delay between collection and mixing with anticoagulant.

Once blood and anticoagulant are mixed, the container should be sealed and mixed by gentle inversion five times. Avoid vigorous shaking. Some authors recommend that the first 5 ml of blood drawn should not be used for tests of hemostasis.

If an evacuated collection system is employed, it should be noted that mixing by five gentle inversions is still required after the blood has been drawn into anticoagulant.

The blood should be mixed with sodium citrate anticoagulant in the proportion 9 parts blood: 1 part anticoagulant. This should be 0.109M (3.2% trisodium citrate dihydrate) or similar (e.g. 0.105M citrate anticoagulant is successfully used in some evacuated systems). Anticoagulant solution can be stored at 4°C for up to three months, but it should be inspected prior to use and discarded if particulate material is present – for example, when contamination has occurred. The sample container should not induce contact activation (i.e. use plastic or siliconized glass). If the patient has a reduced hematocrit, or particularly if the hematocrit is raised, results can be affected. The volume of anticoagulant should be adjusted to take account of the reduced plasma volume. Figure 4.1., below, is a guide to the volume of anticoagulant required for a 5 ml sample.

Figure 4.1. Volumes of blood and anticoagulant required for samples with abnormal hematocrit

Hematocrit	Volume of Anticoagulant	Volume of Blood
25%–55%	0.5 ml	4.5 ml
20%	0.7 ml	4.3 ml
60%	0.4 ml	4.6 ml
70%	0.25 ml	4.75 ml
80%	0.2 ml	4.8 ml

Alternatively, the anticoagulant volume of 0.5 ml can be kept constant and the volume of added blood varied accordingly to the hematocrit. The volume of blood to be added (to 0.5 ml of 0.109M citrate) is calculated from the formula:

$$\frac{60}{100-\text{hematocrit}} \times 4.5$$

CENTRIFUGATION

Platelet-rich plasma (PRP) for platelet function tests is prepared by centrifugation of anti-coagulated blood at room temperature at 150 g–200 g for 10 minutes. The supernatant is removed and kept at room temperature in a stoppered vial during use for a time not exceeding two hours.

Platelet-poor plasma (PPP) is used for most tests of coagulation. The blood sample should be centrifuged at a minimum of 1700 g for at least 10 minutes. This can be at room temperature provided this does not exceed 25°C, in which case a refrigerated (4°C) centrifuge should be used.

Some test procedures require the plasma to be centrifuged twice. To do this, the PPP from the first centrifugation is transferred to a plastic stoppered tube and centrifuged a second time. Care is taken not to use the bottom part of the plasma after the second centrifugation, since it may contain any platelets that remained after the first centrifugation.

SAMPLES FOR IMMEDIATE TESTING

Samples should be tested within four hours of sample collection when possible. More prolonged storage should be avoided for screening tests and clotting factor assays, although it has been shown that whole blood samples stored at room

temperature may be stable for prothrombin time measurements (Baglin and Luddington 1997). Samples for screening tests and assay of factor VII should be maintained at room temperature to avoid the possibility of cold activation.

HIGH-RISK SAMPLES

Care should be taken when handling all plasma samples because of the risk of transmission of hepatitis, HIV, and other viruses.

See Section 2, Laboratory Safety.

DEEP-FREEZING PLASMA

Samples can be stored deep frozen for testing at a later stage. Storage at -70°C or lower is preferable. Clotting factors are stable at this temperature for at least six months (Woodhams et al. 2001). Short-term sample storage at -35°C is adequate for most tests. Storage at -20°C is usually inadequate. Double centrifugation (see Centrifugation, above) should be used if samples are deep-frozen prior to analysis for lupus anticoagulant.

Freezing and thawing is best avoided before APTT determinations, since results obtained by some techniques can be affected. Any frozen plasmas must be transferred immediately to a 37°C water bath, thawed for four to five minutes, and mixed by gentle inversion prior to analysis. A slow thaw at lower temperature should be avoided to prevent the formation of cryoprecipitate, which reduces the FVIII:C, VWF, and fibrinogen content of the supernatant plasma.

REFERENCES

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