

- **REMARK:** Storage of citrated whole blood samples at 2–8°C should be avoided as this may result in loss of FVIII activity.
- **REMARK:** Platelet poor plasma (PPP) samples can be stored at –35°C for up to 3 months and at –70°C for up to 6 months prior to determination of FVIII/FIX activity. Storage of PPP at –20°C is usually inadequate. Freezers with auto-defrost should not be used to store PPP prior to determination of PT, APTT, or FVIII/FIX activity. **CB**

Recommendation 3.2.5:

- The WFH recommends that blood samples for determination of PT, APTT, or FVIII/FIX activity should be rejected and replaced if the collection tube contains less than 80% of the target fill volume.
- **REMARK:** If the collection tube contains between 80% and 90% of its target fill volume, the results obtained using certain methods may have minor artefactual prolongation of PT and APTT and minor artefactual reduction in FVIII/FIX activity. **CB**

Recommendation 3.2.6:

- The WFH recommends that blood samples for determination of APTT or FVIII/FIX activity should be rejected and replaced if in vitro hemolysis or clotting have occurred during the collection and processing of the sample.
- **REMARK:** The impact of in vitro hemolysis on PT is insufficient to affect patient management.
- **REMARK:** Samples from patients with in vivo hemolysis that have been collected for determination of PT, APTT, or FVIII/FIX activity can be accepted and tested. **CB**

Endpoint detection

- Many laboratories now have some form of semi- or fully automated coagulation analyzers. Accurately detecting the clotting endpoint using a manual technique requires considerable expertise, particularly if clotting time is prolonged or if fibrinogen concentration is low, and the clot is thin and wispy.
- For manual testing, the tube should be tilted 3 times every 5 seconds through an angle of approximately 90° during observation. The tube should be immersed in a water bath at 37°C between tilting.

Screening tests

- Platelet count, PT, and APTT may be used to screen a patient suspected of having a bleeding disorder.¹⁹
- The sensitivity of both PT²⁰ and APTT tests^{21,22} to factor deficiencies are influenced by the type of reagents used to perform the test.

Recommendation 3.2.7:

- For laboratory investigation of patients being assessed due to clinical suspicion of hemophilia A, the WFH recommends that prothrombin time testing also be performed using a laboratory reagent containing human tissue factor.

- **REMARK:** Hemophilia A is sometimes excluded despite clinical suspicion of its presence. Such cases may have other factor deficiencies. Some patients with certain FVII defects may have symptoms similar to mild hemophilia but may display normal PT and FVII activity if the laboratory reagent contains non-human tissue factor so that the diagnosis would be missed. **CB**

Recommendation 3.2.8:

- For laboratory investigation of patients being assessed due to clinical suspicion of hemophilia, the WFH recommends that an APTT result within the reference range not be used to rule out the presence of mild hemophilia A or B.
- **REMARK:** In some cases of mild hemophilia A or B, APTT may be within the normal range. **CB**
- Bleeding time testing lacks sensitivity and specificity, and it is also prone to performance-related errors. Therefore, other tests of platelet function such as platelet aggregometry are preferred when available.^{23,24}
- Based on the results of these tests, the category of bleeding disorder may be partially characterized to guide subsequent analysis (see Table 3-1).
- These screening tests may not detect abnormalities in patients with mild bleeding disorders, including some variants of VWD, some cases of genetically confirmed mild hemophilia A or B, defects of platelet function, FXIII deficiency, and those rare defects of fibrinolysis which may be associated with a bleeding tendency.

Correction studies

- Abnormal screening tests may be further investigated using correction or mixing studies.
- Correction or mixing studies using pooled normal plasma (PNP) may help to define whether prolonged coagulation times are due to factor deficiency or circulating anticoagulants or inhibitors.
- The APTT of a patient/normal plasma mix may initially be normal and then progressively prolonged on incubation in the presence of a time-dependent inhibitor (e.g., many acquired autoantibodies against FVIII), although this pattern can be variable in cases with complex kinetics.

TABLE 3-1 Interpretation of screening tests

Possible diagnosis	PT	APTT	Platelet count
Normal	Normal	Normal	Normal
Hemophilia A or B	Normal	Prolonged ^a	Normal
VWD	Normal	Normal or prolonged ^a	Normal or reduced
Platelet defect	Normal	Normal	Normal or reduced

Abbreviations: APTT, activated partial thromboplastin time; PT, prothrombin time; VWD, von Willebrand disease.

^aThe same pattern can occur in the presence of FXI, FXII, prekallikrein, or high molecular weight kininogen deficiencies.