To properly interpret the result of any laboratory test, it is important to have data related to results of the test in healthy normal subjects. Health is not a well-defined condition and is often a relative term. The ideal group in some cases could be closely matched with the population under investigation in respect of age, sex, and, in the case of FVIII/VWF, ABO blood group.

However, such careful selection is not essential for many coagulation tests. In practice, the selection of healthy normal subjects for establishment of a normal range will be influenced by practical considerations. Healthy hospital employees not receiving any medication and healthy blood donors or asymptomatic partners of adult patients under investigation can be successfully used. There are a number of important considerations in relation to normal ranges, which are given below.

The condition of the normal subjects when blood is collected can influence the results obtained. Some of these pre-analytical variables were recently reviewed in an ISTH guideline that related to women’s health issues (Blomback et al. 2007). This includes a review of the evidence for the effects of physical stress (up to 10-hour persistence of a 2.5-fold increase in FVIII/VWF for example), mental stress (increase in FVIII and VWF after acute mental stress), hormone effects, circadian variations, and the effects of posture and diet. Some general recommendations were made, which were not restricted to investigation of female patients. These were as follows:

- Abstain from intense physical exercise for 24 hours prior to venipuncture.
- Use an environment where physical and mental stress are lessened.
- Abstain from fatty foods and smoking on the morning of venipuncture.
- Obtain samples early in the morning (7 a.m. to 9 a.m.), after the subject has sat in a relaxed position for 20 to 30 minutes.

A normal range should always be established locally. Literature and manufacturer’s information should be used only as a guide.

The normal samples should be collected, processed, and analysed using as near as possible identical techniques to that for patient samples.
For screening tests in particular (PT, APTT), the possibility that a new batch/lot of reagent from the same manufacturer has a different normal range than previous similar material should be considered. Internal QC data overlapping any change should be carefully scrutinized. Any changes indicate the need for a different normal range.

For assays, the literature and manufacturer’s information should be used only as a guide. The most suitable assay techniques are those for which the locally established normal range is broadly similar to those in the literature.

Normal ranges of some coagulation tests are different in newborns (pre-term or full-term) and children than in adults (Andrew et al. 1987, 1990, 1992).

Normal ranges, particularly of screening tests, should be used only as an aid to clinical information. Some patients with appropriate personal and family history require further investigations in the presence of normal screening test results. Other patients with abnormal screening tests may not be further investigated where the cause of abnormality is apparent. Normal limits may therefore not be equal to decision and intervention limits.

There are statistical reasons why at least 120 normal subjects are needed to construct a fully valid reference range, but for practical purposes, a close approximation can be obtained by testing a much smaller number, which is considered acceptable for clinical purposes by a number of experts in the field (CLSI 2008). The number of normal subjects selected for analysis should not be less than 30 for tests of hemostasis related to investigation of bleeding disorders.

When constructing normal ranges, the samples from normal subjects should be collected, processed, and analysed locally using identical techniques to those used for the analysis of the patient samples. If the normal practice is for samples to be stored deep-frozen for batch analysis, then this should also be done for normal samples. If patient samples are processed after a delay during which samples are transported to the laboratory over several hours, then a similar delay should be used between collection of samples and testing for the samples from normal subjects used to derive reference intervals. The literature and reagent manufacturer’s information should only be used as a guide.

For each test, the results obtained in samples from healthy, normal subjects are used to construct a normal reference range. The distribution of results of most tests related to investigation of bleeding disorders show a normal or Gaussian distribution. It is useful to confirm this by visual inspection of the data in graphical form, as shown in Figure 8.1. Clear outliers that stand unexpectedly far from most other reference values are probably aberrant results. It is acceptable to exclude these from further calculations.
The frequently used convention is that the reference or normal range should include the central 95% of values. If the distribution differs markedly from that shown in Figure 8.1 — for example, skewed in one direction — additional normal samples may be required. It is convenient to exclude 2.5% of values from either end, leaving the central 95%.

If the distribution is normal, it is appropriate to calculate the mean and standard deviation of the normal values (as described in Section 5), and to use the mean plus 2SD and the mean minus 2SD as the upper and lower limits respectively.

In any case, the normal range should be used only as a guide and aid to clinical interpretation.

For a fuller discussion on establishment of reference ranges, see CLSI (2000).

**Figure 8.1. The distribution of results of APTT in healthy, normal subjects**

Note: The data show a normal distribution, evenly distributed on either side of the mean value.
REFERENCES


Clinical and Laboratory Standards Institute (CLSI). *One-stage prothrombin time (PT) test and activate partial thromboplastin time (APTT) test: Approved guideline*, 2nd ed. 2008; Clinical and Laboratory Standards Institute Document H47-A2.