Von Willebrand Factor Collagen Binding Assay (VWF:CB)

PRINCIPLE
Von Willebrand factor (VWF) has several functions. In addition to being the carrier protein for FVIII in plasma, forming a complex that protects FVIII from proteolysis, it also acts as a mediator for platelet aggregation by attaching itself to platelet membrane receptors (GpIb and GpIIb/IIa) following platelet activation. It is also important in primary hemostasis, acting as a mediator between platelets and the sub-endothelium.

VWF:RCo (Section 29) is a measure of the adhesive properties of VWF but it may not always reflect its physiological function. Measuring the capacity of VWF to bind collagen may sometimes better reflect its physiological function. In most (but not all) cases of VWD, there is concordance between the results of VWF:RCo and VWF:CB. In rare cases of VWD, one of these activities is reduced and the other is within the normal range. Full characterization may require both assays, although many centres use only one of the two. Some authors select VWF:CB in the absence of a suitably precise VWF:RCo.

A number of commercially available kits are on the market at the time of writing. An example is given below, but others are also successfully used. Inclusion of this particular method is not an endorsement of a particular company’s product. If using a different commercial source, it is important to follow the manufacturer’s instructions.

REAGENT
TECHNOZYM VWF:CBA ELISA Kit (Technoclone, Vienna, Austria)
Store at 2°C–8°C, per manufacturer’s instructions.

SAMPLES
Citrated test plasma and calibrators may be stored deep-frozen at temperatures lower than -35°C prior to analysis.

METHOD
1. Ensure kit is brought to room temperature for 30 minutes before starting. The kit can be split into three or four, according to the number of samples to be tested.
Reconstitute standards and control samples with 0.5 ml distilled water and leave for 15 minutes, or thaw if previously frozen. Vortex mix well for 10 seconds.

Dilute all test, control, and standard plasmas 1+ 25 (i.e. 20 μl plasma and 500 μl incubation buffer), then vortex mix.

Aliquot and freeze calibrators and control plasma and store at -80°C.

Add 100 μl of each sample dilution into the appropriate well, cover with film, and incubate for 45 minutes at room temperature (20–25°C). Perform all tests in duplicate.

Prepare washing buffer. Dilute 1 part washing buffer concentrate with 9 parts distilled water and mix well. Prior to dilution, any crystalline precipitates present can be dissolved by incubating at 37°C for 10 minutes.

After 45 minutes, wash three times with 200 μl diluted wash buffer per well, followed by inversion and gentle tapping onto absorbent paper.

Prepare the conjugate working solution by diluting 1 part conjugate with 50 parts incubation buffer. Make up just before use, as it is stable for only 60 minutes. For 8 wells, mix 20 μl conjugate with 1000 μl buffer.

Add 100 μl conjugate working solution to each well, cover, and incubate at room temperature for 45 minutes.

Wash three times with 200 μl wash buffer as before, followed by inversion and gentle tapping onto absorbent paper.

Add 100 μl substrate solution to each well, cover with film, and incubate for 15 minutes at room temperature.

Add 100 μl stop solution to each well.

Shake for 10 seconds. Using a suitable microtitre plate reader, measure the optical densities (OD) within 10 minutes at 450 nm.

Plot (manually or with statistics package) concentration VWF:CB (x axis) against OD (y axis) using linear-linear point to point.

Normal range: 0.49–1.32 IU/ml
FURTHER READING
