Coagulation monitor based on serum migration through absorbent materials

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I describe a method to measure blood coagulation properties, based on the hypothesis that the distance the serum component of a clotted plasma sample moves through a suitable absorbent material should be proportional to the blood's ability to clot. A simple apparatus was constructed to test this principle, in which an absorbent strip contacts clotted plasma samples. At various times, the liquid migration distance into the strip was measured and correlated with clotting times determined by well-accepted procedures. Use of this device to test lyophilized normal and barium-absorbed plasmas, factor VII-deficient plasma, frozen normal plasmas, plasmas from patients undergoing oral anticoagulation, and saline-diluted plasmas (as for Quick Percent assays) showed that clotting times correlated with migration distances for all types of samples ($r^2 = 0.93–0.99$). The device distinguishes between samples from normal and coumadin-treated subjects. The concept can be integrated into an inexpensive, point-of-care coagulation monitor.

Devices to monitor hemostasis and thrombosis range from the manual to the fully automated. Most devices used for coagulation testing determine prothrombin time (PT) or activated partial thromboplastin time, both tests that use a photooptical (or mechanical) clot detector and a timer to measure the time required for a blood sample to clot [1]. Timing begins upon addition of suitable procoagulant chemicals to a blood sample and ends upon detection of the clot. Clotting time devices are used to screen patients before surgery, monitor oral anticoagulant therapy, and diagnose defects in coagulation.

Point-of-care clotting time devices have recently emerged and offer some economic advantages over current laboratory-based devices [2]. The principal advantage is that a central laboratory is not needed in routine use. Another potential advantage is faster turnaround time, which can benefit the patient. Point-of-care devices can also be adapted for home testing—an important consideration in managed healthcare settings. Many point-of-care coagulation devices are simply smaller versions of laboratory-based models, but some point-of-care coagulation devices use novel clot detection and reagent delivery technologies.

In the technology described here, intended for point-of-care testing, serum is formed from plasma after addition of a reagent for PT determination (thromboplastin). After clot formation, an absorbent paper strip is gently placed in contact with the serum-containing clot. Liquid serum migrates from the area of the clot into the absorbent strip. At a defined time, the migration distance is measured and is correlated to the patient's PT.

Materials and Methods

Device construction. A device to test this concept was constructed from common laboratory materials (Fig. 1). A 60-$\mu$L drop of one-stage thromboplastin reagent (see below) was applied to a piece of laboratory film (Parafilm) that was secured to a countertop. The thromboplastin reagent forms a discrete drop on the film surface. A 30-$\mu$L drop of plasma sample was then added to the thromboplastin and allowed to react for ~2 min. With this prototype, the process proceeded at room temperature and no attempt was made to control the temperature of the process; future prototypes will include temperature control. A 5 × 75 mm strip of Schleicher and Schuell no. 903 absorbent paper was gently placed in contact with the drop of plasma/thromboplastin, and the serum in the clotted drop migrated into the absorbent strip. After a time (usually 2 min), the migration position of the liquid front was measured. Generally, the precision in the migration distance was within 2 mm (4%) for an abnormal control.

Plasma and clotting reagents. Thromboplastin reagents included Simplastin® L and Simplastin® HTF, a cultured human cell thromboplastin (both from Organon Teknika), and IL-Test® PT Fibrinogen HS from Instrumentation Laboratory. No differences in migration behavior were
uncovered among the three thromboplastins. Plasma from apparently healthy subjects (“normal”) and plasma from patients stabilized on oral anticoagulant therapy (coumadin) were collected according to routine approved procedures. Coumadin plasma samples from anonymous donors were obtained from Duke University Medical Center.

**Factor VII and saline dilution curves.** A specific factor VII assay was generated by mixing normal pooled plasma in buffered saline with factor VII-deficient plasma (Organon Teknika). A saline dilution curve (as for a Quick Percent assay [3]) was produced by diluting the normal pooled plasma in buffered saline. Specific factor VII and saline-diluted assay samples were analyzed with the method described above and also with an accepted clot-based PT assay, a Coag-A-Mate X2 clotting determined time device (Organon Teknika), according to the manufacturer’s instructions.

**Results**

**Lyophilized controls.** The device was tested with three lyophilized plasma controls (Verify® 1, 2, and 3; Organon Teknika) and one sample from a patient stabilized on oral anticoagulant therapy. Verify 2 and 3 controls are barium-absorbed abnormal controls and Verify 1 is a normal control. The migration distance and the PT time showed a good linear relationship (Fig. 2).

**Factor VII assay curve.** To further assess the behavior of the device, a factor VII assay was generated with saline-diluted normal plasma mixed with factor VII-deficient plasma. Migration distances into the absorbent strip were measured at several endpoints. A nearly linear relationship was found between the migration distance and the percentage of normal plasma for the factor VII curve at each of several migration endpoints (Fig. 3).

**Saline dilution curve.** To assess the behavior of the device in a saline dilution (Quick Percent) assay format, samples of normal plasma were diluted in saline, clotted with thromboplastin, and allowed to absorb into the absorbent paper strip. The PT time was determined with a Coag-A-Mate X2. The Quick Percent assay curve, which relates migration distance to the reciprocal of the percentage of normal plasma in the sample, was linear (Fig. 4).

**Normal and coumadin panel.** To be effective as a screening tool and as a method for monitoring oral anticoagulant therapy, the device must distinguish between normal and abnormal samples. To test the device, migration distances were determined with a panel of normal (n = 6) and coumadin (n = 12) plasma samples. PT values were determined for the same panel. The results showed a good correlation (Fig. 5) between migration distances and PT for both normal and abnormal samples.
For lyophilized normal and abnormal plasma controls, plasma samples deficient in blood coagulation factor VII, saline-diluted normal plasma (as used for the Quick Percent assay), and samples from patients stabilized on oral anticoagulant therapy, the distance of serum migration into an absorbent cotton paper strip is proportional to the samples’ clotting time, as measured on a laboratory clotting time device. Whether the sample’s viscosity, relative serum volume, or hydrophilic character, or a combination of these or other factors, is responsible for the unique behavior observed has not yet been determined.

The current method demonstrates the feasibility of using a coagulation monitor based on serum migration into absorbent materials. The concept could be incorporated into a simple, low-cost, point-of-care coagulation monitor suitable for home-based coagulation monitoring. Future studies will entail refinement of the device and the use of whole-blood samples.

References


Fig. 3. Migration in millimeters vs percentage of normal plasma for a specific factor VII assay. Migration distances into the absorbent strip were measured at 3, 4, 5, and 6 min of migration. The PT value is included for comparison. $R^2 = 0.996$ for the 6-min migration condition.

Fig. 4. Saline dilution (Quick Percent) assay performed with the absorbent paper method. Normal plasma aliquots were diluted in isotonic saline and allowed to react with thromboplastin until clotted. Migration distance for the remaining serum was determined at 3- and 6-min endpoints. PT was determined as described in the text. $R^2 = 0.996$ for the 3-min migration condition.

Fig. 5. Migration distance in millimeters vs PT routinely determined for a panel of normal and abnormal plasma samples (i.e., from patients stabilized on oral anticoagulant therapy). $R^2 = 0.93$ for the entire data set.