Improved Instrumentation for the Determination of Prothrombin Activity

Rex E. Sterling, Alan A. Wilcox, Arnold G. Ware, and Mary K. Umehara

The one-stage prothrombin method of Ware and Stragnell (2) has been semiautomated. This method employs an automatic pipetter-diluter and an end-point detector. These instruments materially increase the output per technician.

Widespread use of oral anticoagulant therapy has made prothrombin time one of the most frequently requested determinations in the clinical laboratory. In order to maintain clinical safety and the effectiveness of anticoagulants it is imperative that a procedure be employed which is maximally reliable and reproducible, and which permits as little variation in individual technique as possible.

The method of Owren (1) as modified originally by Ware and Stragnell (2), and more recently by Ware et al. (3), has been employed in our laboratory for more than 10 years. The technic has been found to be technologically reliable and clinically effective in aiding physicians in the regulation of anticoagulant therapy (4).

This paper presents a method for the semiautomation of the Ware et al. modification in which a diluting apparatus is incorporated to mix plasma and reagents and an end-point detector is employed. These changes have increased the work-output per technician and decreased training time, while maintaining the accuracy and reproducibility of the original manual technic.

Methods and Materials

Reagents and Equipment

Prothrombin-free beef plasma (PFB) This reagent is prepared in our laboratory according to the technic of Ware & Stragnell (2). When prepared in this manner, the PFB may be pipetted in convenient volumes into individual test tubes and stored at \(-20^\circ\), at which it will remain

From the Main Laboratory of the Los Angeles County General Hospital, and the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles 33, Calif. Received for publication April 10, 1964. Accepted for publication June 10, 1964.
stable for at least 6 months. For the final working reagent, it is diluted with an equal amount of 0.45% (w/v) NaCl solution. This dilution is freshly prepared each day, and allowed to stand at room temperature for 1 hr. before use.

*Thromboplastin* To 1 gm. of Bacto-Thromboplastin (Difco Laboratories, Detroit, Mich.) add 56 ml. of 0.9% (w/v) NaCl containing 0.1 ml. of 0.1 M potassium oxalate. This suspension is incubated for 10 min. at 45°, with frequent shaking. After incubation, the mixture is centrifuged at 20 g. for 5 min. to remove coarser particles. At this point, the suspension may be pipetted into individual test tubes and stored at −20°. In the frozen form, it is stable for at least 1 year. The thromboplastin preparation is thawed, diluted with an equal volume of 0.025 M calcium chloride, and incubated for 30 min. at 37° before use. It is kept at 37° while performing the determination.

*Prothrombin standards* The prothrombin standards marketed by Hyland Laboratories have been found satisfactory. The reconstituted vial is used without dilution as the 100% standard. To obtain the 20% standard, 1 ml. of the 100% standard is diluted to a final volume of 5 ml. with distilled water. Standard curves are prepared before the plasma samples are started, near the middle of the run, and at the end of the run.

*Diluting apparatus* The diluter employed is a Dilumat, Model 2 742 (Research Specialties Corp., Richmond, Calif.). The pump sizes are 0.25 ml. for the sample and 1.0 ml. for the PFB. The use of a foot switch for control of the pipetting action substantially reduces the time expended in this operation.

*End-point detector* The B-D Fibrometer (Becton, Dickinson and Company, Rutherford, N. J.) is employed for the detection of clot formation. We have found that 2 of these instruments operating side by side (Fig. 1) significantly increase the speed of operation.

*Anti-coagulant* B-D Vacutainers (Tube 3204X, Formula 17) are employed for drawing the specimen. These tubes contain a stable heparin-oxalate mixture.

**Procedure**

Set the Dilumat sample pump to aspirate approximately 0.010 ml. of plasma. Set the reagent pump to deliver approximately 0.19 ml. of PFB. Connect the clot timer, and allow the temperature to stabilize at 37°. Centrifuge the specimen and place it in a test tube rack, in preparation for performance of the test.

Place the centrifuged specimen under the diluter pipet and aspirate approximately 0.010 ml. of plasma. Return the sample to test tube rack
and wipe pipet tip with a soft cloth or wiping paper. Hold the plastic clot-timer sample cup under the diluter pipet and release the foot switch of the diluter, thereby expelling the plasma sample, and flushing the pipet with approximately 0.19 ml. of PFB. Touch the sample cup to the

![Fig. 1. Arrangement of equipment for determination of prothrombin activity.](image)

pipet tip when removing, to collect any residual drop, and place the cup in the temperature-controlled warming block of the clot timer.

Allow the plasma-PFB mixture to stand in the warming block for approximately 30 sec. Move the cup to the detector well, directly under the detector head. Depress the timer button to clear the timer read-out. Add 0.1 ml. (± 0.02 ml.) of thromboplastin with 0.25 ml. tuberculin-type syringe and simultaneously depress the starting lever. When the clot has formed, the timer and detector will automatically stop. Record the timer reading, lift and turn the detector head, and discard the sample cup. Wipe the electrodes with soft paper. Place the next specimen in the detector well and continue with the next determination.

**Standardization**

Repeat the procedure using the 100% and the 20% standards. The clotting times are plotted on double logarithmic paper, so that patient’s clotting times can be converted to percentages. This technic was described in the original paper by Ware and Stragnell (2).
Discussion

This modification of the method of Ware et al. (3) has been employed for approximately 1 year in this laboratory, and has been found superior to the original manual technic.

No glassware is needed when this method is employed. The pipets used in the dilution of plasma and in the addition of diluted plasma to PFB, as well as the test tubes for the final clotting reaction, may often be sources of error in the manual method. They are very difficult to wash properly, they scratch easily, and the ever-present chipping and breaking of relatively expensive glassware serves to increase substantially the cost per determination.

The use of 0.010 ml. of plasma (as opposed to the 0.10 ml. previously used) makes this technic directly applicable to the determination of prothrombin activity on microspecimens. This simplifies prothrombin determination on infants and newborns, where capillary blood is used routinely.

The incorporation of the end-point detector has virtually eliminated the individual variation in end-point timing previously encountered in the manual technic. It has relieved the technologist from the continuous mental concentration required when he must visually determine clotting times and simultaneously operate stopwatches. The end-point detector has decreased by 75% the time required to train a technician unfamiliar with the technic.

In our laboratory, this system has decreased the time required to perform the daily prothrombin workload of 60–70 specimens, from 4 hr. per day to approximately 2 hr. per day.

By employing 2 of the end-point detectors, as shown in Fig. 1, we are able to perform duplicate determinations simultaneously on each specimen. If the technologist is trained to carry out the dilution step of the procedure on the diluter while the end-point detector is recording the clotting time, the time required per determination is no more than the time required for adding of the thromboplastin, reaching an end-point, and recording the reading.

References