Photoelectric Determination of Plasma Clotting Times

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A photoelectric clot timer is described. The apparatus allows a large analytic capacity, with good precision and correlation to manual methods, and can be used with capillary blood. Prothrombin analysis according to the method of Quick and Owren, the Thrombotest* method, and determinations of partial thromboplastin time are applicable to the apparatus. The semiautomated procedure is extremely simple, and the results are independent of the technicians performing the test.

The determination of plasma clotting times in various modifications has become an important and increasing part of the routine work in the clinical laboratory. The wide application of anticoagulant therapy in clinical surgery and medicine has resulted in many requests for prothrombin determinations. These procedures are a challenge to the capacity and precision of the laboratory and put physical and mental strain on the technicians performing the determinations by manual methods.

Several attempts to automate the determination of clotting times have been made to obtain a simple procedure disengaged from the individual's reflex reaction time and subjective estimate of the point of coagulation, as well as a larger analytic capacity without loss of precision. Two principles have been in use in the development of clot timers: (1) the electro-mechanical principle, where the timer is started manually and stopped by the formation of insoluble fibrin network in the reaction mixture, which serves to complete an electrical circuit through the clot detection system; and (2) the photoelectric principle, where the clotting end-point is assessed by means of the optical changes of the reaction mixture, which occur immediately before the actual clotting is visible to the eye. As in the electro-mechanical systems the timer must be started manually.

Serious objections can be raised against the instruments which have

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appeared on the market so far. The analytic capacity has not been
enlarged—most instruments are only furnished with one or two chan-
nels—and a trained technician can easily analyze two samples at a
time by the manual method. The electro-mechanical instruments work
with electrodes which must be thoroughly cleaned between measure-
ments and which are subjected to continuous mechanical strain. The
precision is hampered by the necessity of starting the timer manually
simultaneously with the addition of the last reagent, and by the fact that
the movable electrode takes 0.5–1.0 sec. to complete a cycle, which is
tantamount to an inevitable variability of the results by 1 sec. Moreover,
some technical mechanical skill of the operator is needed in most electro-
mechanical systems, which is a major disadvantage, as alternating
technicians should be able to operate the instrument day and night. In
the photoelectric systems so far developed, an initial setting of the
instrument after placing the test plasma in the photometric well has
been necessary. The timer must be started and reset manually.

To overcome the disadvantages mentioned, a new semianutomated
clot timer has been developed* which fulfills the basic needs for ca-
pacity, precision, and simplicity. For the reasons mentioned above, the
electro-mechanical procedure was rejected. An analysis of the photo-
electric principle was performed.

To obtain satisfactory electrical impulses for starting the electrical
timer by the addition of the last reagent of the coagulation mixture,
and for stopping the timer by the development of turbidity at the point
of coagulation, the nephelometric response was preferred to the
turbidometric (Fig. 1A).

A photoresistor sensitive to yellow light was selected as photoreceptor
and was included in a voltage divider whereby variation of the in-
tensity of light were changed into variations of voltage, i.e., impulses.
To become independent of irrelevant variations of the background il-
 lumination due to variations of the optical properties of the individual
coaulation mixtures and tubes—and to obtain a high degree of sensi-
tivity and signal-to-noise ratio—the impulses were amplified and
differentiated.

After these steps, the starting impulses are large and of short dura-
tion and may be positive, as well as negative, whereas the stopping
impulses are always smaller, broader, and positive (Fig. 1B). Elec-
tronically, it was now possible to distinguish impulses starting and
stopping the timer by using a triggering system with a start-channel
open to positive, as well as negative, impulses above a certain adjustable

*The clot timer was developed with the technical assistance of Mr. H. Steenbergen from Bie &
Berntsen, Pilestræde 35, Copenhagen, Denmark, who are now manufacturing the apparatus.
minimal value; the instrument is also capable of blocking the stopping channel for an adjustable period of time. The principle of the triggering system is shown diagrammatically in Fig. 2. It soon became evident that the existence of a variable refractory period was a great advantage,

**Fig. 1.** Turbidometric signal as electronic impulses. Abscissa is time, in arbitrary units, and ordinate represents voltage, in arbitrary units. Clotting time of the mixture was 40 sec. Curve B represents the signal before, and Curve A after, differentiation. At $F$ the coagulation process was started by adding CaCl₂, and at $S$ the actual clotting begins.

**Fig. 2.** Principle of triggering system of clot timer.
because addition of CaCl₂ to oxalated plasma caused a "premature" stopping impulse because of precipitation of calcium oxalate. By setting
the refractory period to 5 sec., the influence of this false-stopping impulse was avoided. This may also be accomplished by calibration of
the sensitivity of the stopping channel, which is adjustable within rather
wide limits.

The triggering system described is thus very flexible. The start
channel is open to positive, as well as negative, signals of a magnitude
well above that of the stop signals and is furnished with an adjustable
threshold. The stop channel is open only to negative signals above a
very low and adjustable threshold and is automatically blocked for an
adjustable period of time (the refractory period), which is any time an
impulse passes the starting channel.

The timing system consists of an impulse generator working by di-
vision of the ac frequency separated from the mechanical counter by a
diode gate, which is opened by the starting signals and closed by the
stopping signals. The counters will thus register the time from the addi-
tion of the last reagent until the coagulation begins, i.e., the clotting
time.

The routine model of the clot timer was furnished with six inde-
pendent measuring channels allowing six samples to be analyzed at a
time (Fig. 3). Moreover, the apparatus is furnished with two rows of
six heating wells, and the whole system is thermostated to 37°. With a
preheating time of 2–10 min., it is possible to work continuously with
samples in groups of six with an analytic capacity of 80–100 samples per
hour. The clotting times are read from the mechanical counters in tenths

**Fig. 3.** Clot timer (Prothrombinometer): 1, photometric wells; 2, preheating wells; 3, reservoir for preheating of CaCl₂ solution; 4, thermometer.
of seconds, and the counter is automatically reset when the lid of the photometric well is closed over the next new sample by means of a microswitching system connected to the lid. The tubes used are cheap, disposable standard tubes, and the instrument is independent of variations of the tubes.

**Performance**

**Practicability**

The semiautomated procedure is extremely simple. The coagulation mixtures are prepared, omitting the starting reagent, and are preheated in groups of six for at least 2 min.; they are then transferred to the photometric wells. The lids are closed thus resetting the counters, and the last reagent is added through a canal in the lids; the timers start automatically and run until coagulation starts. The clotting times are read, and the apparatus is then ready for another group of six samples.

**Precision**

The standard deviation calculated from 32 consecutive determinations of clotting times using the prothrombin-proconvertin method of Owren (1) and pooled normal plasma was ± 0.3 sec. with a mean value of 36 sec. Deeply frozen samples from a normal citrated-plasma pool were analyzed on 26 consecutive days and yielded clotting times of 26.6–28.3 sec.

**Correlation to Manual Method**

This has been investigated (1) for the method of Owren and Quick's one-stage test. Blood was withdrawn with 3.8% citrate as anticoagulant and was analyzed by both methods within 2 hr. of collection. The results are well correlated as shown by Fig. 4 and 5.

**Applicability**

The following tests and reagents have been shown to be applicable to the clot timer: prothrombin-proconvertin test; Quick's one-stage test; Thrombotest with plasma (2); and partial thromboplastin-generation test (using "activated cephaloplastin") (3).

**Capacity**

A total of 60–100 samples per hour may be tested.

**Micromethod**

Capillary blood can be used after centrifugation. A series of determinations of the prothrombin concentration using the Owren method on capillary and venous blood from 40 patients showed a good correlation,
with no significant systematic difference between the two methods of blood sampling (Fig. 6). Blood was withdrawn from an ear lobe puncture into a special glass pipet, containing 10 µl of 3.8% sodium citrate, up to the 100-µl mark and was transferred to a microcentrifuge plastic tube and centrifuged (Beckman-Spinco system).

Fig. 4. Correlation between results obtained by clot timer (A) and manual method (B). Prothrombin concentrations were measured according to method of Owren (1).

Fig. 5. Correlation between prothrombin concentrations measured according to method of Quick (1) by clot-timer (A) and manual method (B).
Comment

The clot timer has been in routine use in this laboratory for more than 6 months and has reduced the time needed for the prothrombin determinations from 3 hr. to less than 1 hr. The analytic capacity has been greatly enlarged without loss of precision or simplicity of the analytic procedure.

References