An Evaluation of a Whole Blood Prothrombin Analyzer Designed for Use by Individuals Without Formal Laboratory Training

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Background. The prothrombin time (PT) test, which is the most common coagulation test used in the outpatient setting, has not been recommended for office laboratory use because it has been technically difficult to perform. Consequently, serious errors in patient care could occur because of an erroneous result. The Coumatrak (E.I. Du Pont, Wilmington, Del) now allows nontechnically trained office staff to perform PT tests using a fresh capillary whole blood sample, individually packaged reagent cartridges, and a portable battery-operated instrument.

Methods. Coumatrak PT testing was compared with standard methods for both precision and accuracy, using protocols developed by the National Committee for Clinical Laboratory Standards (NCCLS). Reagent stability and operator variability were also studied.

Results. The results produced by a trained technologist and nontechnically trained staff were comparable. Test results obtained with the Coumatrak were approximately 10% higher than results obtained using standard laboratory equipment and methods using comparable blood samples from the same patients. It was found that the capillary blood specimen had to be rapidly transferred to the reagent cartridge in order to avoid factitiously low results.

Conclusions. The Coumatrak can rapidly provide PT test results that are clinically useful for the office management of patients being treated with a warfarin anticoagulant and for the diagnosis of selected disorders. The system was found to be easy to operate, appropriate for use by individuals with little laboratory experience, and was subject to few operational problems during this study.

Key words. Prothrombin time; comparative study; laboratories; reagent kits, diagnostic. *J Fam Pract 1991; 33:266-271.*

The prothrombin time (PT) test is the most common coagulation test used in the outpatient setting. It can be useful both in the diagnosis of clotting disorders and in the management of patients being treated with a warfarin anticoagulant, which suppresses the production of three of the coagulation factors (II, VII, and X) tested by the PT test.[1] The test determines the time required for initiation of clotting after tissue thromboplastin is mixed with a blood sample. The PT test can be technically difficult to perform even if an instrument to electromechanically or optically determine when a clot begins to form is used, because (1) the reagents have short shelf lives, (2) there is great variability between thromboplastin reagents, (3) the test is sensitive to differences in specimen handling, and (4) the test temperatures must be controlled within one tenth of a degree centigrade. Because of these technical difficulties and the serious patient care consequences associated with an erroneous PT test result, this test has not traditionally been recommended for use in an office laboratory.[2]

A reliable method for determining PT would, however, be very useful in an outpatient practice. This is the standard test used to determine the therapeutic effectiveness of warfarin (Coumadin) anticoagulation. This measure can also be used in the evaluation of patients with a history of abnormal bleeding (especially those with liver disease, malabsorption, or malnutrition) and to assess hepatic function in patients with acute hepatitis. It should be noted that it is neither considered a useful preoperative screening test for healthy patients undergoing surgery, nor a useful test in the screening of otherwise healthy adults.[1] The availability of a reliable PT test

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in an office practice would simplify the monitoring of anticoagulant treatment in office patients with cardiac disease, strokes, recurrent deep venous thrombosis, or pulmonary emboli. It would permit rapid dose adjustments and rapid assessment of the anticoagulation level in patients presenting with bleeding problems (ie, new bruising) while the patient is still in the office.

The PT determination performed in contemporary laboratories involves the use of an instrument that optically or electromechanically monitors clot formation in a plasma sample following addition of thromboplastin and calcium.[3] Because these systems are expensive to acquire and technically demanding to operate, until recently PT testing has been limited to hospital and community laboratories. The Coumatrak is an instrument that determines PT by monitoring the movement of blood through a capillary track in a single-use, disposable test cassette that contains all necessary reagents.[4] The instrument determines the time between application of a fresh whole blood sample and cessation of movement through the capillary track in the cassette. The hand-held Coumatrak is inexpensive to acquire, compared to conventional laboratory instruments, and is designed for use by individuals who have little or no clinical laboratory training.

The purpose of this study was to evaluate the reliability of PT test results obtained by individuals using the Coumatrak who had minimal laboratory training.

Methods

The Analytical System

The Coumatrak system includes a monitor and reagent cassette containing all necessary reagents. The following steps are involved in performing a PT test.

1. The operator activates the monitor by inserting the reagent cartridge, which is then heated to 37°C.
2. The user is then prompted by the instrument display to perform a capillary puncture and to apply a drop of blood to the cassette's sample application site.
3. The blood sample mixes with the reagents as it flows along a capillary path in the cassette.
4. The blood moving in the capillary path is monitored by a laser optical system in the monitor.
5. The PT is determined by the time interval between sample application and cessation of movement of the blood sample in the capillary track.
6. The monitor then displays the determined PT, which has already been adjusted for lot-to-lot variation in reagent activity (self-correcting its calibration electronically).

Coumatrak monitors, electronic controls, normal and abnormal control samples, and Coumatrak reagent cartridges for this study were supplied by the manufacturer. Operation of the instrument was performed according to the manufacturer's directions.[5] Monitors were checked with normal and abnormal quality-control samples each day of the study, and the electronic controls were checked immediately prior to the testing of whole blood precision study or patient samples. Reagent cartridges were inserted into the instrument just before a sample was to be analyzed, and the control or patient sample was applied as soon as the appropriate message appeared on the monitor's display.

Test Protocols

Precision was evaluated using normal and abnormal whole blood controls measured in duplicate twice each study day for 20 days. Two vials of each control level were reconstituted just prior to use, combined in one vial, and refrigerated until used. Ordinarily, a single vial of each level would provide an adequate volume of material for control sample testing. For the precision study, however, which involved four different operators, it was necessary to mix two vials in order to provide an adequate volume for each testing session. All operators obtained their control samples from the same vial and tested these samples within 1 hour of reconstitution. These precision study samples were applied to the Coumatrak reagent cartridge with a plastic Pasteur pipet. One lot of each control level was used throughout the study, but two different reagent lots were required (the second was used only during the last 5 days of the study).

Precision was determined using an adaptation of the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, "User Evaluation of Precision of Clinical Chemistry Devices (EP5-T)."[6]

Result comparability of PT determinations from the Coumatrak was done by testing patient specimens drawn at the same time with the hospital laboratory's MLA 700 coagulation testing instrument using Dade Thromboplastin C reagent.

Specimens were obtained by means of venipuncture of patients for whom a PT had been requested. Although blood from a capillary puncture is ordinarily used with the Coumatrak system, venous blood specimens were used in this study to allow comparison of results produced from essentially the same specimen. Approximately 2 mL of blood was first collected in a vacutainer without anticoagulant to be used with the Coumatrak. A second sample, collected from the same venipuncture in a vacutainer containing a calcium-binding anticoagulant, was sent to the laboratory for a PT determination. A sample was immediately withdrawn from the first vacu-

Table 1. Precision of Prothrombin Time Test Results[6] from Repetitive Testing of Control Materials Using the Coumatrak by Operators Without Formal Laboratory Training

Study Sample	Operator	*n*	Mean	Precision			
				Within Run		Total	
				SD*	CV(%)	SD†	CV(%)
Normal whole blood control	A (MT)‡	79	11.6	0.4	3.63	0.6	5.31
	B	69	12.1	0.5	4.31	0.7	6.11
	C	70	11.6	0.4	3.51	0.5	4.35
	D	57	11.6	0.3	2.95	0.6	4.81
Abnormal whole blood control	A (MT)	79	19.4	1.2	6.31	1.6	8.39
	B	69	19.7	1.3	6.75	1.7	8.46
	C	67	19.2	0.8	4.31	1.6	8.47
	D	58	19.2	1.7	9.07	1.9	10.01

**Manufacturer's product specification[5] = 0.6*
†Manufacturer's product specification[5] = 0.4
‡One medical technologist and three phlebotomists participated in the study; none had prior formal technical training.
n *denotes number of assays; SD, standard deviation; CV, coefficient of variation.*

tainer (with no anticoagulant) using a syringe and an aliquot applied to the Coumatrak reagent cassette. Each operator tested two to three patient samples each study day for 20 working days over a period of 5 weeks (165 patient samples in all). The reagent cartridge lots used in this study were the same as those used in the precision study.

The PT results determined using the Coumatrak were compared with those determined by the hospital's laboratory using an adaptation of the NCCLS "User Comparison of Quantitative Clinical Laboratory Methods Using Patient Samples (EP9-P)."[7]

Control stability was evaluated by testing Coumatrak abnormal whole blood controls that were reconstituted and stored at either room temperature or 37°C for varying times prior to assay.

Reagent cartridge stability was evaluated by testing the normal and abnormal whole blood controls with cassettes that had been stored at 37°C or 4°C for 1 to 26 days. Each day the controls were reconstituted immediately before testing both sets of reagents.

Operators and Operator Training

One medical technologist and three phlebotomists, all of whom had no prior formal technical training, participated in the precision study. Only the phlebotomists tested patient samples in the result comparison study. Each operator was assigned a single Coumatrak monitor and Coumatrak electronic control. Each operator was provided with a one-page summary of operating instructions and given ½ hour of training in the use of the Coumatrak. No further instruction or feedback was given to any of the operators during the balance of the study. This level of training was believed to be typical of the on-the-job training conducted in most office laboratories.

Results

Precision Study

The coefficient of variation (CV) for precision study results using the normal whole blood control material (mean = 11.8 seconds) was between 4.35% to 6.11%, and between 8.39% to 10.01% (Table 1) using the abnormal whole blood control (mean = 19.4 seconds). Mean PT results for the two levels of control material did not differ significantly among the four operators (*t* test, $P < .05$). The within-run precision (CV) and total precision (CV) of the results produced by the medical technologist were essentially the same as the precision of results produced by the phlebotomists (F test, $P < 0.05$).

The variability of control sample results produced by the hospital's clinical laboratory at the time patient samples were analyzed for the accuracy study were CVs of 1.6% (mean = 11.4 seconds) and 2.8% (mean = 19.8 seconds).

Within-run precision using the normal study control sample was less than the manufacturer's specifications for the method (chi-square test, 95% confidence level, Table 1). The total precision of results using normal study control samples and the within-run and total precision of results using the abnormal study control samples were outside the manufacturer's specifications for the method's precision (Table 1).

Five obviously spurious results were produced among the approximately 1000 study control assays that

Table 2. Comparison of Results Produced by the Coumatrak with Those Produced with Comparable Specimens in the Hospital's Laboratory

Operator	Slope	Intercept	Correlation Coefficient	*n*	95% Confidence Interval (secs)
B	1.13	−.4	.95	50	±0.5
C	1.12	−.67	.969	42	±0.6
D	1.10	−.48	.968	63	±0.4

n = number of pairs of results.
Followed adaptation of NCCLS EP9-P protocol.[7]
Least squares analysis.

were performed. These all occurred with testing of the abnormal whole blood control (Lot # CP7E15) and were evenly distributed among the study participants. Each of the operators obtained a result at different times during the precision study that was significantly different from the expected result (20 seconds) for the abnormal control being tested. Three of the operators produced spurious results of 7.6, 12.5, and 12.6 on three separate occasions. The other operator produced results of 29.2 and 30.7 on different days, 2 weeks apart. No similar anomalies were seen with the 165 patient samples tested in the accuracy (method comparison) study, control samples tested as part of the routine daily quality-control testing, or with normal precision study control samples.

Method Comparison Study

Least squares analysis of the patient results produced with the Coumatrak and by the hospital's laboratory was performed (Table 2) and the 95% confidence interval of the average bias for the operator results relative to the laboratory results was calculated.[8] Based on these calculations, a laboratory result of 12 seconds corresponded to mean Coumatrak results of 12.7, 12.8, and 13.1 for the different instruments, while a laboratory result of 20 seconds corresponded to mean Coumatrak results of 21.5, 21.8, and 22.1 (95% CI = ±0.4 to 0.6 seconds) (Table 2).

Reagent Stability Study

Reagent cartridges were stored at either 37°C or in the refrigerator for 1 to 26 days. The manufacturer recommends that the reagents be frozen for routine storage. Normal and abnormal controls were assayed using both sets of these reagents once a day over a 26-day period (Table 3). No difference in either mean values or precision was observed for the two sets of reagent cartridges (*t* test, $P < .05$; F test, $P < .05$).

Table 3. Stability of Reagent Cartridges*

Reagent Cartridge Storage Temperature	Normal Control Results			Abnormal Control Results		
	Mean	SD	*n*	Mean	SD	*n*
4°C	11.5	.15	26	20.5	.24	26
37°C	11.6	.12	26	20.9	.23	26

**Control Lot # CP7A01; Cartridge Lot # P702B17.*
Note: Controls assayed each day over a 26-day period.

Sample Timing Study

Finger-stick blood samples from a single patient were assayed either immediately or after a 10-second delay (Table 4). The mean value for the immediate assay results (12.97 seconds) was significantly longer than the mean value for the delayed assay results (11.17 seconds [*t* test, $P < .05$]).

Discussion

Results produced with the Coumatrak were approximately 10% higher than results obtained from a sample collected from the patient at the same time but tested in the hospital's laboratory with conventional equipment and methods (Table 2). Prothrombin test standardization has been a longstanding problem because of the variability of different instrument and reagent systems[9] and the multiplicity of reporting methods. A PT test result in one laboratory cannot, therefore, be equated with the same result determined using a different instrument or reagent system in another laboratory.[9] The use of the International Normalized Ratio (INR) in reporting PT test results is now recommended to neutralize result differences between different instruments or reagent systems.[10] The ratio of the patient's PT test result to the normal PT test result is corrected using the International Sensitivity Index (ISI) for the specific thromboplastin used in the instrument or reagent system employed to produce the result. The Coumatrak monitors used in the study had the capability of reporting INR results but the clinical laboratory in the University Hospital (Portland, Oregon) was not reporting the INR value at the time this study was performed. Had such a

Table 4. Change in Prothrombin Time When Sample Transfer Is Delayed 10 Seconds

Sample	Sample Results*		
	Mean	SD	*n*
Immediate assay	12.97	.82	21
10-second delay	11.17	.63	18

**Results were determined from blood samples from the same patient taken at the same time.*

ratio been used in this study, it is possible that the apparent difference between the Coumatrak and the reference PT results might have been reduced. For clinical purposes, the practitioner using the Coumatrak system needs to be cognizant of the correlation between its results and those determined by other laboratories in their community. To address this issue, one hospital (VA Medical Center, Portland, Oregon) has initiated an additional quality-control measure that requires each operator to obtain a venous sample after every tenth Coumatrak determination. This sample is sent for testing to their clinical laboratory (which now reports both conventional and INR values). The results of both analyses are then evaluated for excessive variance.

The precision of results produced with the Coumatrak by a medical technologist and by briefly trained nonlaboratorian operators was comparable (Table 1). However, the variability of the study control sample results produced by all operators was greater than the total precision specifications given by the manufacturer (Table 1) and greater than the variability of PT control sample results produced with automated equipment in the hospital's laboratory. The protocol used in the precision study[6] was designed to provide an index of true day-to-day result variability in actual use. The variability found with this protocol[8] is usually much higher than that found with less demanding methods used primarily to validate the methodology.[4] The variability of results produced in the sample timing study (doing repetitive PT testing in a single individual with a normal prothrombin test result) was comparable to that found in the precision study using study control samples (Table 4).

The Coumatrak was simple to operate and seemed appropriate for use by individuals with limited technical background and minimal training. Instructional prompts are provided by the instrument to guide the operator through each step of the procedure. A finger on the reagent cartridge is pointed at the target-like area where the sample needs to be applied. The Coumatrak has a built-in fail-safe system to detect whether an inadequate volume of blood has been applied to the reagent cartridge. If a "short sample" is detected, a message to the operator appears on the display indicating that the analysis is not valid and should be repeated. Initially, users accustomed to performing capillary blood glucose testing applied an inadequate volume of blood to the cartridge, resulting in the need to perform the test a second time. Once the operators became aware of the volume of capillary blood required, there generally was no problem with obtaining and applying an adequate volume of blood to the cartridge.

One important operational factor found to affect the accuracy of PT results produced with the Coumatrak system involved the delay in sample application to the reagent cartridge initiating the testing process. If transfer of the capillary blood sample from the puncture site on the finger to the reagent cartridge is delayed for 10 seconds, the average value of PT test results is 10% lower than results obtained for samples that were tested immediately (Table 4). Since test timing begins when the sample is applied to the reagent cartridge, it seems obvious that delayed transfer allows the clotting process to begin before the sample is applied to the reagent cartridge. Any significant delay in transferring a patient's capillary blood sample to the reagent cartridge to start the testing process could, therefore, result in factitiously low PT results. This problem can be avoided if the sample is promptly transferred to the reagent cartridge. Since the Coumatrak requires that the operator open the reagent package, insert the reagent cartridge into the instrument, and allow time for the cartridge to warm up before the specimen can be applied, it is essential that the operator be familiar with the timing of the required steps prior to testing patient blood samples.

Another potential problem was the five obvously spurious results obtained during the precision study. The frequency of these spurious results was low (0.5%). An inquiry into the reason for these results revealed that the operators did not adequately mix the normal and abnormal controls during those assays. There were no obvious outliers when the Coumatrak results were compared with the results determined by the clinical laboratory using standard methods for the same patient. A manufacturer's representative indicated that the spurious results found in the precision study, using whole blood control material as the study sample, may be caused by infrequent problems with the reagent cartridge—control interaction. The problem was evident only with the abnormal study control sample and was not seen in routine control sample testing for daily quality-control purposes, in the precision study using the normal study control material, or in any of the patient sample results in the accuracy study.

Except for questions raised by the spurious control results described above, the control materials and reagent cartridges performed acceptably. There was no significant change in PT results produced with reagent cartridges that had been stored for up to 26 days at 37°C compared with cartridges that had been stored for the same length of time in a refrigerator (4°C). In portable instruments such as the Coumatrak, reagent stability is essential because the instruments are likely to be used in a wide range of health care settings.

At the time this report was being written, the ability to perform PT testing outside conventional clinical laboratories was being evaluated, and may be affected by the final regulations (not yet published) implementing the

Clinical Laboratory Improvement Amendments of 1988. In the initial draft regulations, the PT test was classified as a complex test that could be performed only by fully qualified technologists. Because of the magnitude of the negative response to the published draft regulations, the Health Care Financing Administration has indicated that it will revise the classification of tests and testing devices covered by the law. There is reasonable probability that the Coumatrak and other simple one-step test devices will be reclassified to permit their use by nontechnically trained individuals.

The Coumatrak can rapidly provide PT test results that are clinically useful for the office management of patients being treated with a warfarin anticoagulant and for the diagnosis of selected bleeding disorders. The system was easy to operate, appropriate for use by individuals with little laboratory experience, and few operational problems were encountered during this study.

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