Optical and Mechanical Clot Detection Methodologies: A Comparison Study for Routine Coagulation Testing

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Background: Automated coagulation analyzers are preferred to meet increasing coagulation test volume. Two distinct technological families exist based on optical and mechanical clot detection methodologies. Which one is superior to the other is still a conflict and needs new studies. **Methods:** We have compared prothrombin and activated partial thromboplastin results obtained with mechanical method with those obtained by photo-optical method used routinely in our specialized

laboratory. **Results:** The instrumental results showed good precision ranging between 0.7% and 1.8% coefficient of variation. Statistical analysis demonstrated an excellent correlation between the photo-optical and mechanical analyzers for PT (R^2 0.97), and aPTT (R^2 0.85). **Conclusion:** Correlation between the two clot-detection systems was maintained even when measuring turbid samples ($R^2 \geq 0.97$ for two tests). J. Clin. Lab. Anal. 26:125–129, 2012. © 2012 Wiley Periodicals, Inc.

Key words: blood coagulation; factors; partial thromboplastin time; prothrombin time; clot detection; mechanical detection; optical detection

INTRODUCTION

Coagulation analyzers run the gamut of options, from automated instruments to manual devices, from high-volume processing to low-volume analysis, and from a wide selection of assays to a small, core group of tests. Because of the increasing demand for high-volume, routine coagulation testing, automated coagulation analyzers have become more popular over the last decade. Examples of automated instrument include the Coag-A-Mate MTX-2 (MTX II) and AMAX 200 (1, 2). Trinity-Biotech's MTX II (TrinityBiotech, Berkeley Heights, NJ) is a medium-volume analyzer with many of the features of high-volume systems. Using photo-optical detection with a 405-nm halogen light source, the compact, bench top analyzer performs many tests besides basal coagulation tests, including: prothrombin time (PT) and activated partial thromboplastin time (aPTT). AMAX 200 (Trini-

tyBiotech) can measure clot formation in both photo-optical and mechanical modes. It has been widely held that mechanical clot detection is unaffected by turbid samples and, hence, is superior to photo-optical detection, which, in contrast, may be affected by turbid samples (4–7). This has led to the belief that mechanical detection provides a true clotting time (CT) determination for coagulation testing. Some studies have suggested that optical and mechanical detection methods are equivalent in terms of correlation, accuracy, and precision for coagulation testing, and that both methods are unaffected by sample turbidity (8–10). Other studies have suggested that optical detection is superior to mechanical detection (8–10), such as in a clinically important case of dysfibrinogenemia caused by a familial mutation (11) and a biphasic waveform that indicated a suspicious sepsis patient (10). Consequently, the advantage of one detection method over the other remains unknown. In the present study, we set out to obtain new information on the comparability of core haemostasis assays in routine patient samples in a high-volume hospital setting. The PT and aPTT tests were performed in both the optical (MTX II) and mechanical (AMAX 200) modes and the results were compared using linear regression. Within-run, total precision and reference intervals according to relevant NCCLS guidelines were

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also determined for each assay on the MTX II and AMAX 200 (12–17).

MATERIALS AND METHODS

The randomly-selected patient samples obtained from the normal course of the clinical laboratory's daily routine were prospectively collected into a tube (Becton Dickinson light blue tube) containing 3.2% sodium citrate and tested for PT and aPTT. MTX II, an automated photo-optical coagulation analyzer, was one of the test methods. The optical measurement was based on the change of physical aspect of the plasma (because the fibrin polymer will catch some proteins and reduce the opacity of the sample). When measured optically, a significant difference of absorbancy of the sample was observed after the clot formation. The AMAX 200, an automated photo-mechanical coagulation analyzer, served as the comparative method. The mechanical measurement was based on the true clot detection; the analyzer is using the ball to detect the presence of the clot in the reaction cuvette. Both systems are distributed by TrinityBiotech, Berkeley Heights, New Jersey, USA. To reduce variability, only TrinityBiotech reagents, controls, calibrators, and consumables were used on the two test systems. A total of 424 patient samples were included in the study during a period of 15 days. In cases where the sample was hemolyzed or icteric or lipaemic, the sample's physical aspect before the test was changed. The physical aspect of each sample was done and turbid or hemolyzed samples were classified as inappropriate.

Plasma from 25 normal healthy individuals was assayed to obtain the geometric means and reference ranges for PT and aPTT for both the MTX II and AMAX 200 instruments, respectively. Daily, weekly, and monthly quality control and maintenance were performed following the manufacturers' recommended procedures.

STATISTICAL METHODS

Mean, standard deviation (SD) and percentage coefficient of variation were calculated for each assay. Calculations were performed by linear regression analysis with

an $R^2 > 0.95$ predetermined as an acceptable correlation. Statistical significance was defined by a hypothesis test yielding a P -value of less than 0.05.

RESULTS

Reference ranges and precision

Plasma samples from 25 healthy normal donors yielded the following reference ranges: for PT, 10.5–12.3 s and 11.3–13.4 s, respectively, for MTX II and AMAX 200; for aPTT, 24.5–33.9 s and 25.5–35.8 s, respectively, for MTX II and AMAX 200. Both normal and abnormal controls demonstrated excellent interassay and intraassay precisions for two assays well within manufacturer's specifications.

Sample summary

We randomly collected 424 clinical samples from a routine high-volume clinical laboratory. As shown in Table 1, among the 424 samples, 420 (99%) had PT results, and 388 (91.5%) had aPTT results. Thirty-two samples had visually observed interferences due to haemolysis or lipemia.

Correlation between photo-optical and electromechanical clot detection

Correlation was determined using more than 400 clinical samples over a 15-day time period. Statistical analysis demonstrated an excellent correlation between the photo-optical and mechanical analyzers for PT ($R^2 0.97$), and aPTT ($R^2 0.85$) Table 1; Figs 1, 2.

"No clot" samples

For two assays examined, any result that showed "no clot" was either printed as "no clot found" (MTX II), or "no coagulation" or "analysis time over" (AMAX 200). Although a total of 424 samples were tested, some were not included in the data set due to inappropriate volume for one of the two methods. For PT, among the 412 samples tested, one sample yielded a "no clot" error on MTX

TABLE 1. Intraassay Precision of MTX II and AMAX 200 in Normal and Abnormal Control Products ($n = 20$)

	Normal control				Abnormal control			
	PT(s)		aPTT(s)		PT(s)		aPTT(s)	
	MTX II	AMAX 200	MTX II	AMAX 200	MTX II	AMAX 200	MTX II	AMAX 200
Mean	11.3	12.1	26.2	25.9	38.3	41.8	537	63.2
SD	0.1	0.1	0.1	0.1	0.3	0.8	1.2	0.7
CV (%)	0.6	0.9	0.5	0.5	0.7	1.8	1.8	1.2

Fig. 1. Correlation of TriniClot PT HTF performed on MTX II and AMAX 200 ($n = 420$).

Fig. 2. Correlation of TriniClot aPTT S performed on MTX II and AMAX 200 ($n = 407$).

II due to the fact that the upper limit of the PT setting is 100 s on MTX II, whereas it produced results by AMAX 200 (130.4 s). For aPTT, 388 samples were tested, with five samples showing “no clot” on MTX II, whereas they provided results with AMAX 200. As shown in Table 4, in no case did MTX II provide a PT or an aPTT result, whereas AMAX 200 yielded “no coagulation.”

Samples with visually observed interferences

A total of 32 samples (2.8% overall) with visually observed interferences were identified and tested on MTX II

and AMAX 200 for PT and aPTT. Further analysis of the subgroup of turbid samples indicated that the correlation remained strong between the two detection methods (PT ($R^2 0.97$), and aPTT ($R^2 0.98$)) (Table 6).

DISCUSSION

Our study was designed to obtain new information on the comparability of mechanical and photo-optical detection methods in determining CT for a given sample. Current results were similar to other studies that photo-optical and mechanical clot detections are satisfactory

TABLE 2. Interassay Precision of MTX II and AMAX 200 in Normal and Abnormal Control Products ($n = 30$)

	Normal control				Abnormal control			
	PT(s)		aPTT(s)		PT(s)		aPTT(s)	
	MTX II	AMAX 200	MTX II	AMAX 200	MTX II	AMAX 200	MTX II	AMAX 200
Mean	10.4	9.3	28.2	31.1	45.9	49.5	65.5	75.2
SD	0.1	0.2	0.4	0.6	2.0	1.5	2.5	1.5
CV (%)	1.4	1.8	1.5	1.9	4.3	3.0	3.8	2.0

TABLE 3. Reference Values. 95% Confidence Intervals are Shown in Parentheses. Reference Values are for 25 Healthy Volunteers

	Median (ranges)		Reference value, mean (SD)	
	MTX II	AMAX 200	MTX II	AMAX 200
PT (s)	9.8 (6.6–100.0)	10.6 (8.0–104.8)	10.5–12.3	11.3–13.4
aPTT (s)	28.6 (17.7–199.2)	29.4 (20.8–208.2)	24.5–33.9	25.5–35.8

TABLE 4. Number of “No Clot” Sample Results by Test Method

method Test	Total sample size ^a	No clot on MTX II and reported result on AMAX 200	Excluded for hemolysis, lipemia, or icterus
PT (s)	412	1 (0.2%)	15 (0.9%)
aPTT (s)	388	5 (1.2%)	17 (4.38%)

^aVarying sample sizes were due to lack of clot formation in certain assays.

for the basal coagulation not only in samples with normal optical quality but also in optically abnormal specimens (e.g., in lipemia, hyperbilirubinemia, and haemolysis) (18). Photo-optical and mechanical detection for routine coagulation analysis (PT and aPTT) were highly correlated in 412 samples tested over a 15-day time period in a high-volume clinical laboratory (Table 5). Concerning intra- and interassay precision, CVs were below 5% in both assays. We also observed that the two methods generated similar reference intervals and SDs. A small percentage of normal results as not measured by MTX II were reported on AMAX 200 (0.2 and 1.2 PT and aPTT, respectively) (Table 4). In clinical practice, proper reference ranges for PT and aPTT are typically established with a local normal donor group. In establishing such reference ranges, certain diagnostic criteria must be met, including sensitivity for various factor assays related to the “intrinsic” and “extrinsic” pathways to assure adequate differentiation of normal coagulation from underlying factor deficiencies when obtaining an abnormal CT (3).

Our results indicate that turbidity has no significant effect on photo-optical detection as compared with mechanical detection. We have shown equivalent results between the two systems with 32 turbid samples. The issue of sample integrity for coagulation testing continues to be

TABLE 5. Correlation Analysis for All Samples Performed on MTX II and AMAX 200

	Correlation (<i>r</i>)	Slope (95% CI)	Intercept (95% CI)	Comparison (<i>P</i>)
PT (s)	0.97	1.033	(–) 0.43	<0.01
APTT (s)	0.85	0.96	(–) 0.87	<0.01

TABLE 6. Correlation Analysis for 32 Turbid Samples Tested on MTX II and AMAX 200

	Correlation (<i>r</i>)	Slope (95% CI)	Intercept (95% CI)	Comparison (<i>P</i>)
PT (s)	0.97	0.96	0.85	<0.001
APTT (s)	0.98	0.96	0.89	<0.001

a growing concern in clinical laboratory practice due to preanalytical error caused by the blood collection personnel with varied backgrounds and experience. Particularly, haemolysis during the blood collection process must be identified and those samples replaced with nonhemolyzed samples when possible (19, 20).

Regarding setting criteria to perform alternative procedures, our study demonstrated equivalency between photo-optical and mechanical detection methods. It is therefore reasonable that one method may be used for the other when considering repeat testing.

CONCLUSION

All data obtained during this study indicate that the patient results obtained by the photo-optical detection system are as reliable and statistically equivalent as those obtained using the mechanical detection system. Most notably, we also found that when testing suboptimal samples (i.e., hyperbilirubinemia, lipemia, etc.) the photo-optical and mechanical detection methods are statistically equivalent.

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