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Comparison of Photo-Optical and Mechanical Methods for Prothrombin Time Test

KEYWORDS: prothrombin time; mechanical method, photo-optical method, automation, coagulation

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ABSTRACT *Background: The PT measures the activity of the so-called extrinsic and common pathways of coagulation. Automated coagulation analyzers have replaced the manual methods to meet the increasing test load in many laboratories. Two distinct automated or semi-automated methods exist based on optical and mechanical clot detection.*

Aim: To compare the reliability of prothrombin time test using mechanical and photo-optical methods in a semi-automated coagulation analyzer.

Materials and methods: Prothrombin time data was collected retrospectively conducted on 100 samples run on mechanical & optical modes on Amax Destiny Plus™ analyzer (Trinity Biotech) in the month of November 2012. The standard deviation (SD), Coefficient of Variance (C.V.) & R^2 value were calculated.

Results: The instrument results showed good precision and coefficient of variation. Statistical analysis demonstrated an excellent correlation between the photo-optical and mechanical methods for PT (R^2 0.995).

Conclusion: The results for the prothrombin time test obtained by the photo-optical detection method is reliable and is statistically equivalent as those obtained by the mechanical detection method

- **Introduction:**

The prothrombin time was first described by Quick in 1935 and the test was often referred to by the eponym 'Quick's Prothrombin Time(Quick ,*et* al 1935) The prothrombin time was developed to measure Prothrombin (Factor II) and hence bears its current name. However, it subsequently became clear that it was sensitive to abnormalities of factors VII, X, V, II and fibrinogen. The prothrombin time is a one-stage test based upon the time required for a fibrin clot to form after the addition of tissue thromboplastin, phospholipid and calcium to decalcified platelet poor plasma. The manual methods have little value in large clinical laboratory of a tertiary care hospital which caters to hundreds of samples each day.

Automated and semi-automated coagulation analyzers have replaced the manual methods to meet the increasing test load. The modern analyzers adopt different technologies to measure the prothrombin time. The advantages conferred include minimal manual interference, increased precision, reduced manpower and a large number of samples can be processed to meet the ever-increasing sample load. Of the different methods employed in this regard, two distinct methods exist which are utilized in most laboratories. They are based on the principles of photo-optical and mechanical clot detection(Bai, *et al* 2008)

- **Photo-optical method:**

Detection of clot formation measured by a change in optical density (OD) of a test sample. As the plasma sample clots, it becomes more optically dense & the amount of light falling on a photo-sensitive detector decreases (i.e. transmitted light decreases).The drop or change in light is determined as the endpoint.

- **Mechanical method:**

Clot detection involves monitoring the movement of a steel ball within the test solution using a magnetic sensor. As clot formation occurs, the movement of the ball changes, which is detected by the sensor

In our current laboratory set-up, we test hundreds of samples each day on a semi-automated coagulation analyzer (Amax Destiny Plus™ -Trinity Biotech) . Hence, we decided to compare the reliability of prothrombin time test using mechanical and photo-optical methods in a semi-automated coagulation analyzer.

- **Materials and Methods:**

The design of the study was retrospective type. Data was collected from 100 samples of prothrombin time test in the month of November 2012 in the Clinical Laboratory and Hematology division of Kasturba Hospital, Manipal after receiving a clearance certificate from the Hospital Ethics Committee.

The samples for prothrombin time (PT) testing were collected in a BD™ vacutainers containing 0.109M 3.2% sodium citrate as anticoagulant. As per the laboratory protocol, we run the sample first in the mechanical mode to record the prothrombin time (PT) on the Amax Destiny Plus™ semi-automated analyzer. The samples with a PT exceeding 16 seconds are run on the photo-optical mode in the same machine. We selected 100 such samples which were run on both modes. The lipemic, hemolyzed and turbid samples were excluded from the study. The sample results were charted in a tabular format on Microsoft Excel spread-sheet™ for further analysis.

The standard deviation (SD), Coefficient of Variance (C.V.) & R^2 value were also calculated from the data collected. Calcu-

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lations were performed by linear regression analysis with R^2 >0.95 as an acceptable correlation.

We ensured the internal quality control of the instrument by running two level controls (abnormal high and normal) thrice each day.

- **Results:**

The PT ranges with their corresponding C.V. are summarized in table 1

The instrument results showed good precision and coefficient of variation over different ranges of PT. We consider a CV less than 3 as acceptable.

The statistical analysis demonstrated an excellent correlation between the photo-optical and mechanical methods for PT (R^2 0.995) (figure 1)

- **Discussion:**

Coagulation testing is an important part of every medical practice. Every laboratory is usually inundated with requests for prothrombin time (PT) and activated partial thromboplastin time (APTT). The prothrombin time is a one-stage test based upon the time required for a fibrin clot to form after the addition of tissue thromboplastin, phospholipid and calcium to decalcified platelet poor plasma. Ever since its successful description by Quick in the early twentieth century, the manual testing of PT has stood the test of time. But the place of the manual method in a large clinical laboratory of a tertiary care hospital; which caters to hundreds of samples each day has been eclipsed by automated methods. These instruments analyze the coagulation system through detection of clot formation (Ens GE.,*et al* 1993; Sabo 1982) By the 1960s, semi-automated coagulation instruments based on electromechanical methods and optical methods of clot detection were in vogue. These instruments have led to an increased precision and accuracy and, therefore, improved diagnostic testing and monitoring of therapeutic interventions.

The two main methodologies have been widely popular. We currently use Amax Destiny Plus™ (Trinity Biotech) semi-automated analyzer in our laboratory; which has both modes. However, the photo-optical methods have distinct drawbacks such as a spuriously increased PT in case of turbid and lipemic samples where the optical density is increased. There have been studies comparing both methods, which claim the superiority of mechanical method when these variables are present (Quehenberger *et al* 1999 ; Fischer *et al* 2006).

Other studies uphold photo-optical method especially in dysfibrinogenemia and sepsis, where derangements in fibrin and fibrin degradation products can produce unreliable results using the mechanical method (Lefkowitz *et al* 2000; Toh *et al* 2003).Consequently, the advantage of one detection method over the other remains mired in speculation. Very few studies in published literature have concentrated on these differences of these methodologies. Hence, we proceeded to test the reliability of both methods on the same test sample.

In our laboratory, a PT value more than 16.0 seconds (done on mechanical mode) is repeated with the optical method to rule out a possibility of a random error or a spurious variable. In this study, we noticed that PT results across different ranges by both modes yielded good and acceptable Coefficient of Variance (C.V.). In a recent large scale clinical study, Tekkesin and colleagues performing standard coagulation testing on more than 2,000 clinical samples in a tertiary care hospital (Tekkesin N *et a*l 2012). Our results were similar to theirs with respect to a high correlation between both methods for PT. (R^2 =0.995 present study vs. R2=0.96). They also found an excellent correlation between the two clot detection systems even when measuring turbid samples (R^2= 0.98 for all assays). This aspect was not evaluated by us due to paucity of such samples.

- **Conclusion:**

The result for the prothrombin time test obtained by the photo-optical detection method is reliable and is statistically equivalent as those obtained by the mechanical detection method. Both methodologies can be relied upon while testing for coagulation.

Table 1: Average CV for different PT ranges:

PT range (seconds)	Average CV
16.0 – 20.5	0.00
20.6 -30.5	0.83
30.6 – 40.5	2.51
40.6 – 50.5	1.57
>50.5	2.82

Figure 1:

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