Research Article

A comparison study of routine coagulation screening tests (PT and APTT) by three automated coagulation analyzers

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Received November 13, 2015. Accepted November 26, 2015

Abstract

Background: In recent times, growing coagulation test volume and constricted personnel budgets have enhanced interest in automated coagulation analyzers.

Objective: (i) To compare the reliability of routine coagulation test [prothrombin time (PT) and activated partial thromboplastin time (APTT)] using mechanical, photo-optical, and nephelemetric methods by three automated coagulation analyzers. (ii) To evaluate the performance of a newly installed fully automatic coagulation analyzer [STA Compact Max (Stago)] and compare the consistency of its testing results with the confirmed clinical automatic coagulation analyzer at our department (Sysmex and ACL Top).

Materials and Methods: Trisodium citrated (3.2%) 60 blood samples, which came to special (coagulation) laboratory with request of PT and APTT (with or without anticoagulant, abnormal, and normal/controls), Transfusion Medicine and Immunohematology Department, Christian Medical College, Vellore, Tamil Nadu, India, were included into study randomly. Sample were run on Sysmex CS2000i (Sysmex Corporation, Japan), photo-optical clot detection; ACL Top (Instrumentation Laboratory, USA), nephelometry clot detection method; and STA Compact Max (Stago, USA), viscosity-based (mechanical) clot detection.

Result: Correlation was determined using Bland and Altman analysis, which demonstrated a good agreement between Sysmex CS2000i, ACL Top, and STA Compact Max for PT and APTT. A total of 10 samples (16.7%) with visually observed interferences were identified and tested on all three analyzers and showed good agreement with optical and mechanical methods.

Conclusion: This study showed good agreement between newly installed STA Compact Max based on mechanical endpoint detection method with already standardized Sysmex CS 2000i and ACL Top, which work on photo-optical endpoint detection method, for evaluation of screening coagulation tests such as PT and APTT even in case of variables such as partially lysed/icteric samples. Three automated analyzers can be used interchangeably.

KEY WORDS: Coagulation, automated analyzer, coagulation screening tests

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2016.13112015254	

Introduction

In 1935, Armand Quick[1] demonstrated prothrombin time (PT), a clot-based test of the extrinsic and common coagulation pathways. The PT is sensitive to decreased levels of factors VII, X, V, and II and fibrinogen. Factor VII deficiency affects PT most sensitively, whereas factor II (prothrombin) and fibrinogen deficiencies exhibit least effects. Its chief

applications are in screening inherited and acquired coagulation disorders and monitoring vitamin K-antagonist treatment. The addition of thromboplastin, which comprises tissue factor plus phospholipids, along with the addition of calcium chloride initiate the PT test.[2] The time taken for a fibrin clot formation is then determined by either an optical or a mechanical method. The unit of measurement of PT results is seconds, with a classic range of approximately 10–13 s.

The activated partial thromboplastin time (APTT) is a clot-based test of the intrinsic and common coagulation pathways. It is generally used in screening inherited and acquired coagulation disorders and monitoring unfractionated heparin treatment. In 1953, Langdell et al.[3] devised it as PTT, where the phospholipid and calcium chloride activated the coagulation.[3] Proctor and Rappaport demonstrated the activated PTT (APTT) alteration in 1961 by activating the contact factors with kaolin. The name of the test derives from the use of a partial thromboplastin or procoagulant phospholipid that activates the clotting mechanism; whereas thromboplastin is a complex of tissue factor and phospholipid.

Proenzymes characteristically existing as inactive in the intravascular space along with cofactors, cations, and cell-associated phospholipids comprise the coagulation system. The two chief mechanisms, intrinsic and extrinsic pathways, which merge to yield thrombin using a common pathway characterized by a series of interrelated enzymatic reactions, activate the coagulation.[4,5]

These classical pathways form the basis of the two most frequently performed coagulation tests: the PT, which measures the extrinsic and common pathways, and the APTT, which measures the intrinsic and common pathways. Nonetheless, the physiologic activation of coagulation in vivo is not so separated, with the initiation stage taking place via tissue factor exposed during vascular injury, resulting in a consequent propagation stage, and added amplification of the process by thrombin, owing to the activation of factors V, VIII, and XI.[6–8]

The APTT is performed in two stages. In the first stage, the APTT reagent comprising a standardized amount of procoagulant phospholipids and contact activator is added to citrated anticoagulated plasma. After a standard incubation time, calcium chloride is added and the clotting time measured.[8] The activator differs but is generally kaolin, silica, ellagic acid, or any other negatively charged substance.

In recent times, growing coagulation test volume and constricted personnel budgets have enhanced interest in automated coagulation analyzers. These coagulation instruments carry out in vitro tests that enable determination of hemostatic defects and monitoring anticoagulant treatment. Most of the tests (e.g., PT and PTT) are based on identification of a fibrin clot as the endpoint. Fully automated coagulation analyzers can automatically deliver reagents and plasma samples to the reaction cuvette, barcode sample detection, execute dilutions and computer data storage of patient samples, and control results and calibration curves.[9]

The tilt tube method forms the basic method for clot-based coagulation, in which the plasma and reagents are pipetted into a clear test tube by the operator, a timer is started, and the back and forth tilting of tube until a clot forms is performed, at which point the operator stops the timer. Although rarely used nowadays, it still forms the gold standard for comparison of instrument results.

Our aims for study were

1. To compare the reliability of routine coagulation test (PT and APTT) using mechanical, photo-optical, and nephelometric methods by three automated coagulation analyzers.
2. To evaluate the performance of a newly installed fully automatic coagulation analyzer STA Compact Max and compare the consistency of its testing results with the confirmed clinical automatic coagulation analyzer at our department (Sysmex CS 2000i and ACL Top).

In automated and semi-automated optical instruments, plasma becomes turbid or opaque owing to fibrin formation, and two methods are used in detection of clot formation: mechanical detection based on electromechanical and electromagnetic properties and optical method based on photo-optical and photometric properties.

Electromechanical: A fibrin strand completing an electrical circuit forms the basis. A probe with two electrodes with current passing between them is dropped into a cup with plasma and reagents. The fibrin detected between the electrodes results in a detection circuit that senses the finished circuit, which is the endpoint (fibrometer).

Electromagnetic mechanical: A raise in plasma viscosity when a fibrin forms is the basis of the technique. A steel ball is oscillated within a cuvette under an electromagnetic field and monitored. Clotting of the plasma sample slowdowns the ball movement, which forms the endpoint. Interference by lipemia and bilirubinemia with the results attained using mechanical detection should not occur.

Photooptical: Fibrin strand formation scatters the light forms the basis. Clot formation in the plasma sample clots makes it optically denser and reduces the quantity of light falling on a photo-sensitive detector (i.e., transmitted light reduces). The reduction or alteration in light is considered as the endpoint.

Photometric: Occurrence of absorbency (optical density) of a monochromatic light (uses filter) passing via the cuvette as the reaction being determined forms the basis. Measurement of transmitted light and conversion to absorbance results in the determination of the substance concentration. Lipemia, icterus, and hemolysis may interfere the optical instuments.[10]

Materials and Methods

Sixty whole blood samples were collected into trisodium citrate (3.2%), which came to Hemostasis Laboratory with request of PT and APTT (with or without anticoagulant, abnormal, normal/controls, and icteric/lysed), Transfusion Medicine

and Immunohematology Department, Christian Medical College (CMC), Vellore, Tamil Nadu, India, were included into study randomly. Study grant was approved by institutional review board and ethical committee, CMC, Vellore. All samples were analyzed on Sysmex CS2000i (Sysmex Corporation, Japan), photo-optical clot detection; ACL Top (Instrumentation Laboratory Company, USA), nephelometry method; and STA Compact Max (Stago, USA), viscosity-based (mechanical) clot detection.

Results

We randomly collected 60 samples from the routine hemostasis laboratory, which included 20 normal, 10 partially lysed and icteric, and 30 abnormal (with or without anticoagulant) samples [Table 1]. Correlation was determined using Bland and Altman analysis, which demonstrated a good agreement between Sysmex CS2000i, ACL Top, and Stago Compact Max for PT and APTT [Tables 2 and 3]. Difference plot of Sysmex vs. Max showed abnormal PT (0.10%) and abnormal APTT (0.03%) results that were above the agreement line. Difference plot for ACL Top vs. Max showed abnormal APTT (0.03%) and partial lysed/icteric APTT (0.1%) results that were above the agreement line [Figures 1 and 2].

Table 1: Sample details

60 samples (PT and APTT)	
Normal	20
Abnormal	30
Partially lysed/icteric	10

Of 60 samples, few samples showed no clot detection because of upper limit of clot detection. Six samples from ACL Top, two samples from Compact, and two samples from Sysmex showed no clot detection for APTT. One sample from each analyzer showed no clot detection for PT.

A total of 10 samples (16.7% overall) with visually observed interferences were identified and tested on all three analyzers and showed good agreement with optical and mechanical methods (reference ranges: PT, 10–12.5 s; APTT, 25.1–36.7 s).

Discussion

In recent times, growing coagulation test volume and constricted personnel budgets have enhanced interest in automated coagulation analyzers. These coagulation instruments carry out in vitro tests that enable determination of hemostatic defects and monitoring anticoagulant treatment. Most of the tests (e.g., PT and PTT) are based on identification of a fibrin clot as the endpoint. In automated and semi-automated optical instruments, plasma becomes turbid or opaque owing to fibrin formation, and two methods such as photo-optical and mechanical methods are used in detection of clot formation.

Study was designed to compare newly installed automated coagulation analyzer STA Compact Max in our Hemostasis

Table 2: PT Sysmex vs. Max and ACL vs. Max

PT	Average difference	SD	Limits of agreement
Sysmex vs. Max			
Normal	–0.770	0.181	–1.125, –0.415
Abnormal	–1.234	1.074	–3.340, 0.871
Partially lysed/icteric	–1.580	0.464	–2.489, –0.671
ACL vs. Max			
Normal	0.335	0.499	–0.642, 1.312
Abnormal	0.383	1.072	–1.719, 2.485
Partially lysed/icteric	–0.770	1.316	–3.349, 1.809

Table 3: APTT Sysmex vs. Max and ACL vs. Max

APTT	Average difference	SD	Limits of agreement
Sysmex vs. Max			
Normal	–5.230	2.745	–10.610, 0.150
Abnormal	–1.721	17.212	–35.455, 32.013
Partially lysed/icteric	–6.040	12.718	–30.968, 18.888
ACL vs. Max			
Normal	–4.225	2.659	–9.437, 0.987
Abnormal	12.832	10.543	–7.832, 33.496
Partially lysed/icteric	–1.940	5.663	–13.040, 9.160

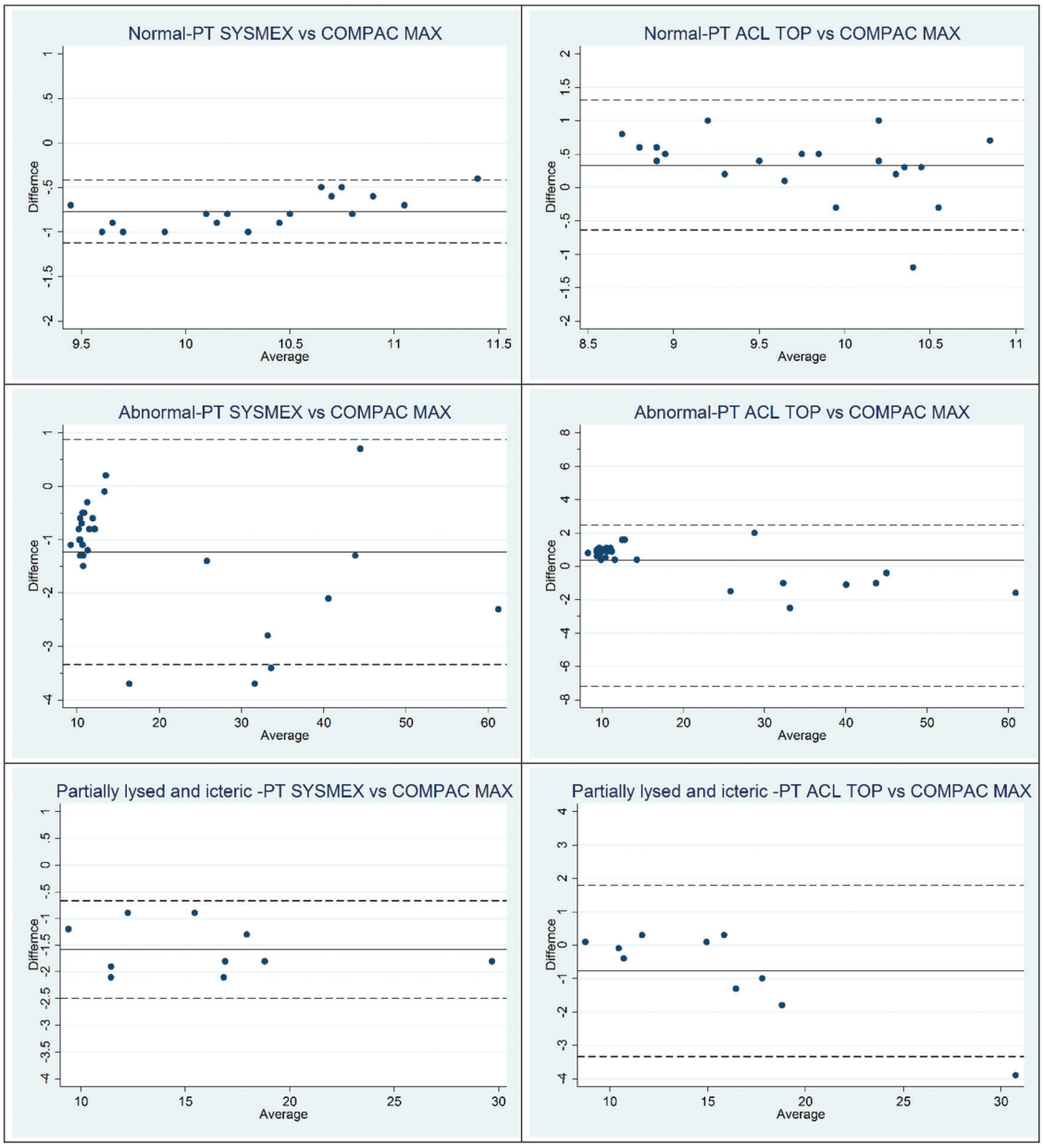

Figure 1: Bland and Altman plot for PT (Sysmex, ACL, vs. Compact Max).

Laboratory in which the endpoint detection is by viscoelasticity-based mechanical method for routine coagulation screening tests such as PT and APTT with already standardized current analyzers Sysmex CS2000i (photo-optical method) and ACL Top (nephelometric principle). Totally, 60 samples including 20 normal, 10 partially lysed/icteric, and 30 abnormal high samples with or without anticoagulant were analyzed on three different analyzers, which work on different principles to check the reliability of PT and APTT. Reference range for PT and APTT, which was validated by laboratory, was used for all three analyzers.

Analysis done by Bland and Altman difference plot[11] showed good agreement between the all three automated coagulation analyzers, which was similar to that observed by Tekkesin and Kılınc.[12] and other studies.[13–15] Difference plot of Sysmex vs. Max showed abnormal PT (0.10%) and abnormal APTT (0.03%) results that were above the agreement line. Difference plot for ACL Top vs. Max showed abnormal APTT (0.03%) and partial lysed/icteric APTT (0.1%) results that were above the agreement line. So, we can use this method interchangeably whenever required such as when there is a high value.

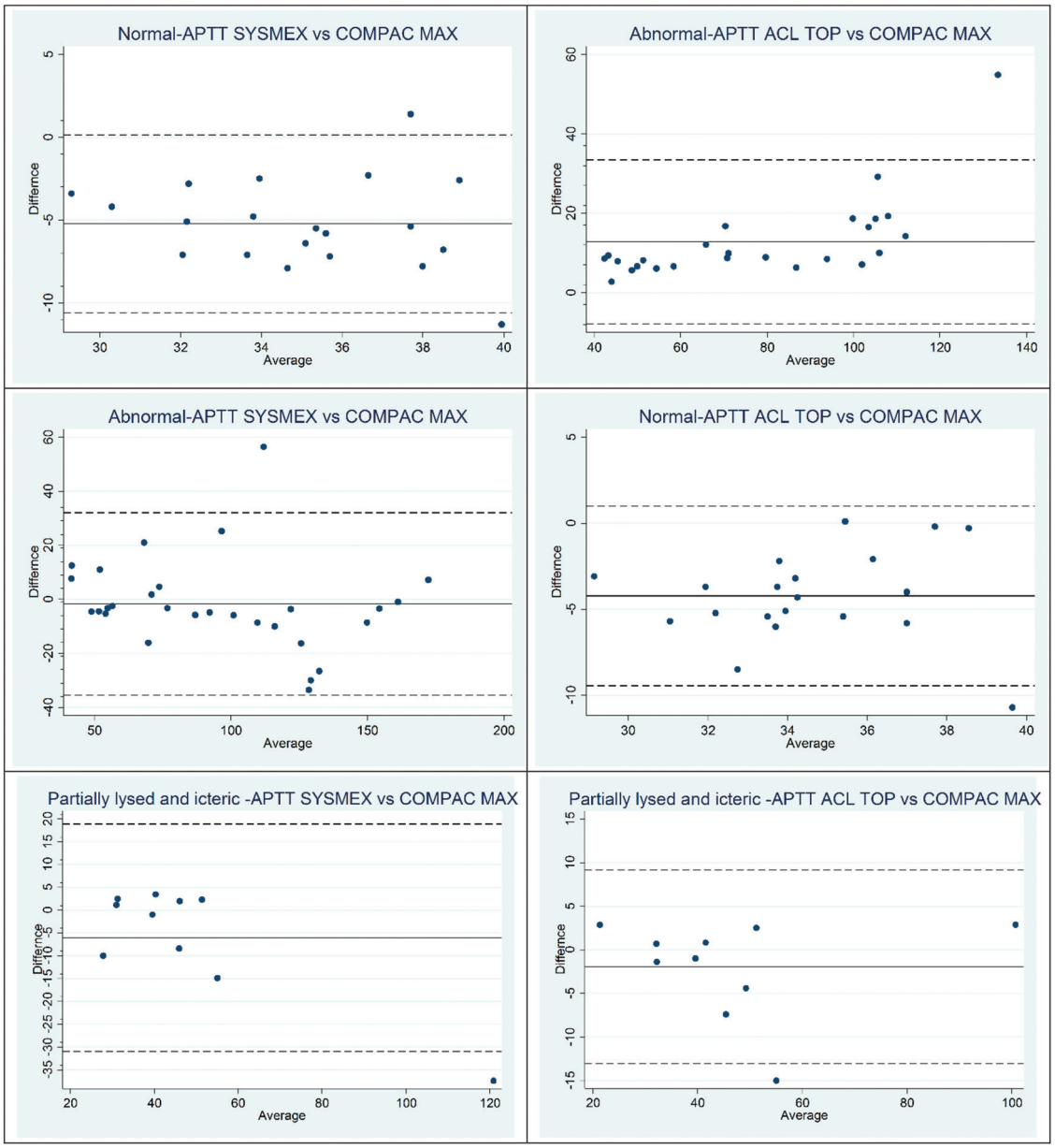

Figure 2: Bland and Altman plot for APTT (Sysmex, ACL, vs. Compact Max).

In case of partially lysed/icteric sample, there was a good agreement among the different analyzers, which is opposite to study that claimed the superiority of mechanical method when these variables were present (Quehenberger et al. 1999; Fischer et al. 2006). One of the reason for this maybe because new analyzer possessed HIL (hemolysis, icterus, and lipemia) detection system using multiwavelength light. A preanalytical check for interfering substances or less number of samples with all these variables is required.

Six samples for APTT from ACL Top showed no clot detection but clot was detected in Compact Max and Sysmex.

As, according to our laboratory protocol, all hemolyzed samples should be replaced by new fresh samples, we could not study those samples.

Conclusion

This study showed good agreement between newly installed STA Compact Max based on mechanical endpoint detection method with already standardized Sysmex CS 2000i and ACL Top, which work on photo-optical endpoint detection

method for evaluation of screening coagulation tests such as PT and APTT. The agreement between three automated coagulation analyzers was consistent in normal sample, abnormal samples, and in cases of interfering substances such as partially lysed/icteric samples.(HIL). Three automated coagulation analyzers can be used interchangeably when needed.

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How to cite this article: Rathod NN, Nair SC, Mammen J, Singh S. A comparison study of routine coagulation screening tests (PT and APTT) by three automated coagulation analyzers. Int J Med Sci Public Health 2016;5:1563-1568

Source of Support: Nil, **Conflict of Interest:** None declared.