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Assessment of Measurement of Prothrombin Time using Different manufactured Prothrombin Reagents Used in Khartoum State Laboratories

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Abstract:

Introduction: *This study has been done during the period from March to July 2012 which-was carried out in the central laboratories building of Al-Zaiem Al-Azhari University and Khartoum Teaching Hospital.*

The study aimed to evaluate the measurement, accuracy and precision of the common PT reagents coming from different companies (TEclot, futura System, Stago and Fortress) used in Khartoum state laboratories.

Materials and Methods: *This study was descriptive comparative study done in (twenty-nine) patients under oral anticoagulant therapy taken from Sudan Heart Center Hospital for comparing if there are difference of results between the four companies, using Quick and Owren manual method. In addition to human control sera which obtained from Technoclot Company for evaluating their quality in accuracy and precision according to reference result, using automated (sysemx ca-500)*

Results: *This study showed that PT reagents have different result for the same patient's samples either in control sera the reading showed statistical difference of the PT result between all the companies except between Stago company and TEclot company which there are no significant difference. Furthermore calculation of the INR patient's*

results showed there are significant difference between all companies expect TEclot Company and fortress In addition our Results showed the most accurate company is Fortress (INR=1), and the other companies gave acceptable results according to reference Value (0.91 - 1.09) expect futura System Company which have significant difference to control value (1.16)

***Discussion**: This study showed there are statistical difference between PT reagents manufacture d by different companies which apparently agreed with previous studies of Nancy Grove study, Kristine Rotzoll study The University of Iowa Hygienic Laboratory. Also we found that there was the average of accuracy and precision differ between all companies.*

Key words: Prothrombin Time, Prothrombin Reagents, Khartoum State Laboratories

1- Introduction and Literature Review

1.1 Quality:

Laboratory quality is the basis of correct medical interpretation of laboratory result. Quality control/ quality assurance refers to all these planned and systematic actions required to provide adequate confidence that a result, product or service satisfies the given requirements for quality. This well-known process is only part of an overall quality system. This comprises organizational structure, responsibility, procedures, processes and resources for implementing quality management. Quality standards in laboratory diagnostic tests basically depend on the quality requirements defined by the medical issue under investigation. (1)

1.2 Precision:

Precision means the degree of agreement of repeated management of a quality. It is indicated as imprecision, i.e., the

scattering of measured result (standard deviation) around the mean, and is expressed as coefficient of variation to improve overall comparability. Precision is the first and most important component of the quality of laboratory result.

Coefficients of variation of less than 5% are prescribed for most laboratory methods. Coefficient of less than 2% can often be achieved using modern automated procedures. When establishing methods, the first step is to run a test for interaserial precision. This can also be carried out in the form of scattering of duplicate determinations. In this case, standard deviation is calculated as follow:

$$S = \sqrt{\frac{\sum \Delta^2}{n-1}}$$

$\sum \Delta^2$= sum of squares of differences (= x -x^-)

S=mean value, x=result, n=number of duplicate determinations

The reproducibility limit according to ISO is: An expected value that is less than or equal to the absolute difference between tow single results of measurement, obtained under the conditions specified from reproducibility of measurement results (with probability of 0.05) (1)

1.3 Accuracy

Accuracy refers to closeness with which measure value agree with true value. Inaccuracy is defined as the difference - μ where μ is the true value or a reference value. A t-test can be performed to determine if the difference is statistically significant:

$$t = \frac{\bar{x} - \mu}{s} . n$$

1.4 Preanalytical Phase:

The term "phase" includes all steps that a test sample use undergoes until the actual test is carried out. They include:

sample collection, labeling, transport (and storage, if applicable), pretreatment and evaluation of the test sample. (1)

1.5 Control Material/ Reference Material:

Quality testing materials are used in different ways and may be called control material if they are used in process control. They may be called reference material if their nominal values are of especially high quality. Calibration materials are often of the same natural as control materials. But they are used in another way. There is basically no difference between the material themselves when a quality testing material is used for process control or external quality assessment, but the technical requirements for Process control materials are often less stringent because they are only intended for use with a single method for each component.

In any case, the characteristic target quantity should be in sufficiently stable form in the control material, and the composition of the sample material should comparable to that of control material. (2)

1.6 Internal Quality Control:

Internal laboratory quality control comprises the control of imprecision and inaccuracy. Although the investigator knows the concentration when checking imprecision, he does not know the concentration when checking accuracy. The goals of precision and accuracy controls are different as well.

The internal laboratory quality control is the indispensable basis of all quality assurance actions carried out in the medical laboratory, and makes possible an immediate decision regarding the release of a series of analyses, or compels one to repeat a series, check the analytical system, or even to interrupt work until be problems have been clarified.(2)

1.7 The quality control of the Prothrombin time:

The quality control of the Prothrombin time has two main aspects: quality control of production of the national reagent, and quality control of performance at individual hospitals. The quality control of production of the standardized thromboplastin was based originally on the development of a method for the large scale production of a working thromboplastin preparation identical between batches.

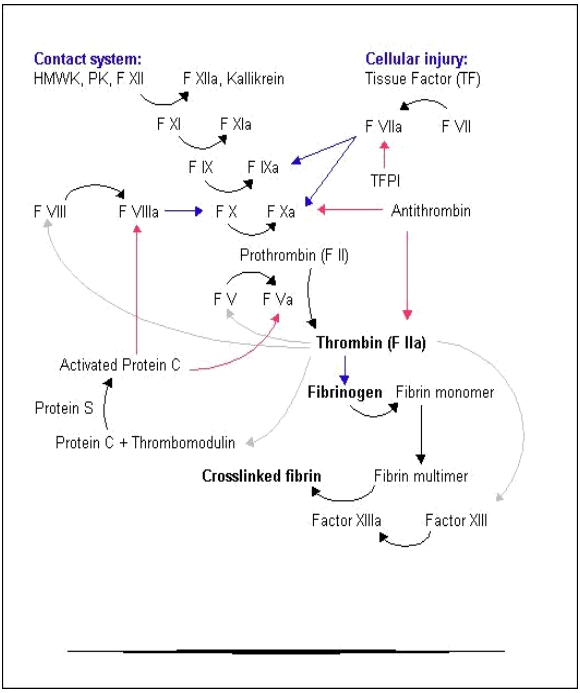
Special problems of quality control of clotting tests arise from the fact that human clotting proteins are labile. Some deteriorate rapidly, whereas others are subject to activation and increase of activity on storage. Unless infinite care is taken in laboratory preparation of such quality control samples, to overcome the effects of activation by storage and processing and the effects of deterioration, the vials of material produced-will are subject to lack of uniformity and lack of stability. Improved techniques have enabled us to produce normal and abnormal plasma samples free from interval variation and with good stability and storage after reconstitution. The following pattern has evolved. After an advance warning, a set of three abnormal lyophilized plasmas, together with standardized thromboplastin and calcium chloride for the recalcification of the plasma samples are supplied to each hospital. They are also given an instruction sheet with the recommended technique and a form for the return of the results. (3)

1.8 Current Model of blood coagulation:

The arrest of bleeding involves complicated process in the body. The characterization of coagulation pathways and coagulation factors has constituted an enormous undertaking, lasting over two hundred years since Hewson (1780) made the first observation in fibrinogen (coagulation factor I). For hemostasis two major components are necessary: a platelet plug and a stable fibrin clot. The latter can be formed in vivo through an

"intrinsic" or an "extrinsic" pathway. The final outcomes of both pathways are the same, i.e. the reaction from coagulation factor X to insoluble fibrin. The bio-chemical structures of coagulation proteins as well as the structure and code inheritance of the respective genes are for the most part known. (3)

Figure (1-1). Schematic illustration of the "intrinsic pathway" and "extrinsic coagulation pathway"



Injury and exposure to foreign surface under vascular endothelial cell (surface activator) with exogenous phospholipid and starts the "intrinsic coagulation" and the subsequent reaction produce an insoluble fibrin clot. The activated partial thromboplastin time (APTT) test is sensitive to decreased blood levels of coagulation factors in the "intrinsic pathway".

Abbreviations:

Table (1-1): The intrinsic pathway's factors

Factor Abb	Descriptive name	Active form
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
PK	Prekallikrein	Serine protease
HMWK	High molecular-weight kininogen	Cofactor
PL	Phospholipid	

Injury, cell breakdown (tissue thromboplastin, exogenous phospholipid, Ca^{+}) initiates the "extrinsic pathway" reaction and the end product is an insoluble fibrin clot.

Abbreviations:

Table (1-2). The extrinsic pathway's factors

Factor Abb	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/ cofactor
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
X	Stuart-Prower factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutaminase
PL	Phospholipid	
Ca^{+}	Ionized calcium, factor IV	

Factor IXa can bind to the cofactor factor VIIIa bound on membrane surface in the presence of calcium ions to generate a complex with enzymatic activity known as tenase. This complex converts the proenzyme factor X to its enzyme form, factor Xa.
In a parallel series of interactions, factor Xa bind to the cofactor factor Va, bound on membrane surfaces, in the presence of calcium ions to generate a complex with enzy-matic activity known as prothrombinase. (4)

1.9 Development of Modern PT Analysis:

Warner and colleagues (1934) and Quick (1935) developed independently and published at almost the same time the first techniques for measurement of certain plasma coagulation factors. They designated the test Prothrombin time, (PT). In the Finnish terminology the term "thromboplastin time" (TT) is used instead of PT, and is employed throughout the present work.

In the method developed by Warner and associates also called the low Two-stage Test, the two phases of clotting (Prothrombin to thrombin and fibrinogen to fibrin) are separated and the reaction of fibrinogen to fibrin measured. The estimation was technically more complicated than that for Quick's Prothrombin Time and is no longer in clinical use.

In the Quick Prothrombin time an excess of thromboplastin and calcium is added to oxalate-anticoagulanted plasma. The clotting time was considered to be direct measure of the Prothrombin concentration of the blood (Quick 1935).

The Quick reagents currently used are based on this principle. The test is easy to perform both manually and automatically. The principle basis of the PT test for laboratory and Point-of-Care Testing (POCT) methods is presented in Figure 2.

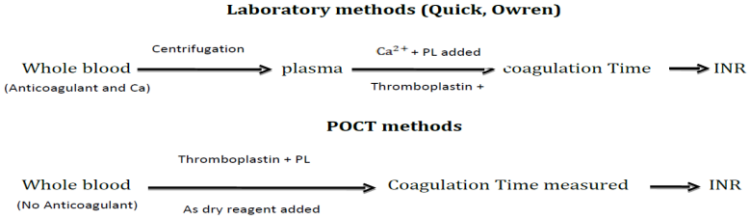


Figure (1-2). Basic model for PT measurement using laboratory or POCT method.

PT and APTT measurements have also recently been adopted in Point-of Care Testing (POCT) technology. These methods are now wide in clinical use and are targets of general interest. In this case laboratory technology has come close to the patient, offering new possibilities for patient care also in anticoagulant therapy monitoring. Patient can perform by the test at home (Price and Hicks 1999). (4)

1.10 Prothrombin Time:

The Prothrombin time test measures coagulation factors of the "extrinsic pathway" illustrated in Figure 1. It is carried out by adding thromboplastin (containing tissue factor). Phospholipid and an excess of calcium to anticoagulanted plasma and measuring the clotting time. PT is the most commonly used coagulation test in routine laboratories.

The PT test is used as a pre-surgical screening tool for inherited or acquired deficiencies of the extrinsic pathways of coagulation. It is also the most common test used to monitor patients receiving anticoagulation therapy. An abnormal PT may indicate: Vitamin K deficiency, severe liver disease and Deficiency in various blood clotting factors.

The PT test is used for assessment in pre-operative detection of bleeding tendencies in risk groups, the monitoring of anticoagulant therapy; used for prevention and treatment of venous thromboembolism, prosthetic heart valves, arterial fibrillation and other indications. The most markedly increasing use of PT is now seen in oral anticoagulant therapy (OAT) control. The need for PT tests is constantly increasing as the population ages and more thrombotic diseases are encountered. PT determinations during OAT are needed at regular intervals since the therapeutic range of oral anticoagulant is narrow. Aged patients are in greater danger of bleeding: over-medication increases the bleeding risk and under-medication entails the possibility of thrombosis.

OAT inhibits the synthesis of coagulation factors II, VII and X in the liver. In addition, the vitamin k antagonists limit the carboxylation of the proteins C and S and impair their function. So most PT determinations are used for monitoring the efficiency of OAT. (4)

1.10.1 Sampling:

- *Anticoagulants in sampling for coagulation tests:*

Manyanticoagulants are available for blood sampling for clinical tests. One group of them bind and inactive calcium. These include, for example, citrate, EDTA and oxalate.

Ionized calcium (factor IV) is in key position in both the intrinsic and the extrinsic coagulation pathway. When anticoagulants bind (inactive) calcium in blood sampling, they simultaneously inhibit coagulation reaction in the test tube and preserve coagulation factors for further analyses. In the coagulation activity measurements, excess calcium is added to set off the coagulation reaction.

For anticoagulation of blood samples for coagulation analysis the World Health Organization (WHO) and the National Committee for Clinical Laboratory Standards (NCCLS) recommend the use of 3.2% (0,109 mol/I) citrate and dilution of 1 part of citrate with 9 parts of blood for coagulation test sampling (WHO 1999). Unfortunately this recommendation is not necessarily carefully followed and the citrate concentration used may thus vary. This causes problems in analytical accuracy and precision, resulting in variability of PT result between different laboratories.

The Quick PT method is highly sensitive to differences in sample citrate concentration and this may cause analytical errors both in INR result and in ISI standardization, the proportion of citrate in the final sample is not constant: although constant in the test tube, it is distributed only in the plasma phase of the blood sample. Blood hematocrit has an

important effect on the citrate concentration: high hematocrit means a high citrate concentration (small volume of plasma) and low hematocrit means a lower citrate concentration (in a larger volume of plasma). Owing to the relatively large proportion of citrate in the final analysis, the Quick PT method is sensitive to sample citrate concentration, and this constitutes one important source of analytical variation. (5)

- *Sampling and other preanalytical preparations for PT:*

The accuracy of the Prothrombin time result, as in all other coagulation tests, depends greatly on the quality of sampling. Venous compression before vein puncture should be no more than 60 seconds. Underfilling of specimen tubes causes a higher citrate concentration in the sample (plasma) and this comprises a source of error. Tubes should be filled to at least 90% of capacity to avoid falsely elevated PT coagulation times. Tissue contamination of the sample should be minimal, in POCT instruments capillary blood from the fingertip without exogenous anticoagulant is usually used for analyzing PT. Plasma or whole blood collected with citrate, oxalate or EDTA as anticoagulant are incompatible with most POCT instrument. (5)

- *Sample stability:*

All published works on sample stability have been conclude using the Quick PT method, but they are obviously also relevant for the Owren PT, since more coagulation factors are measured with Quick PT (factor I, II, V, VII and X) than Owren PT (factors II, VII and X)(8)

Sample tubes should be stored at room temperature, the reason being that factors VII activated and the PT becomes shorter at 4^0C in both whole blood and plasma when stored in borosilicate or siliconised borosilicate tubes. Recommendations in the literature are variable regarding the maximum duration

of plasma storage before PT measurement. PT should be estimated within two hours at room temperature or within four hours at 4^0C from sample collection or eight hours at room temperature.

Baglin and Luddington (1997) reported that plasma is highly stable in unopened sample tubes; the overall mean difference in INR after three days was only 0.05 INR units as compared to a fresh sample.

Leeming and associates (1998) noted that the mean PT change after 24 h was relatively small, but that some sample changed markedly, namely over 0.5 INR units, which is too much for clinical purposes. (10)

1.10.2 Units of Prothrombin Time Measurement;

1- Time (seconds):

Modern handbooks of clinical chemistry give PT reference values only in seconds. Depending on the thromboplastin reagent, values of 10- 13 s (Henry 2001) and 10- 12 s (recombinant thromboplastin) as well as 11- 16 s (Lewis 2001) have been presented. These reference intervals are for the Quick method; Owren coagulation times are longer, since the relationship between sample and reagent is different. The corresponding reference interval for Owren reagents is 17.4-22.6 s.

Using a second as unit there is no need for calibration, which is an advantage, since calibration may cause variation and errors in result. The disadvantage is that PT coagulation times vary according to the reagent source and even form batch to batch. Furthermore, the coagulation time is highly dependent on the source of thromboplastin raw material, and the therapeutic time range for OAT depends on the reagent.

When seconds are used as unit, only one measurement and no calibration is required. This automatically results a

smaller variation and better accuracy as compared with ratio and INR strategies. (11)

2- Ratio and percentage:

Since the second as unit is highly dependent on reagent PT units. Ratio (R) and percentage (%) units were adopted for clinical use according to the following formulas:

$$\textbf{Eq.a R} = \frac{Sample\ Sec}{Normal\ Plasma\ Sec} \quad \textbf{E.q.b \%} = \frac{Sample\ Sec}{Normal\ Plasma\ Sec} \times \mathbf{100}$$

It is obvious that R and % values were introduced in clinical practice in order to reduce differences between different reagents and to harmonies the therapeutic range for OAT. Biggs and Denson (1967) proposed standardization towards an international reference thromboplastin and the ratio method was accepted by WHO (1977). (12)

3- INR (International Normalized Ratio):

WHO has supported the use of INR units and reference thromboplastin globally. This way done in order to harmonies PT result in OAT monitoring and to facilitate the comparability of related scientific work. WHO furthermore recommends editors and reviewers of scientific paper not to accept manuscripts expressing their PT result in traditional terms without INR values. Recommendations for therapeutic ranges for different patient groups are now globally uniform as INR units. (14)

INR units were originally not recommended for screening and follow-up of patients with liver diseases. However, Kovacs and associates (1994) have since demonstrated that INR can also be used to monitor patients with severe form of liver disease. The use of INR has encountered many unpredictable difficulties and much remains

to be done in order to harmonise results globally, as in necessary to increase the safety of patients on OAT. However, Hirsh and colleagues (1998) claim that the INR system is much more reliable and clinically safer than PT in seconds or the unconverted PT ratio for monitoring OAT.(13)

A computer simulation study of serial INR measurements has been conducted within the most widely used therapeutic range (INR 2.0 – 3.0); the authors concluded that analytical imprecision should be <5% and analytical bias <پ} 0.2 INR units.

Mathematics of INR calculation:

The Following formula is used for INR calculation:

$$\mathbf{INR} = \left(\frac{Sample\ Sec}{Normal\ Sec}\right)^{\mathrm{ISI}}$$

The equation for calculation of PT INR values is designed to eliminate the variance caused by reagents and instrumentation. The INR calculation formula is based on the previously used ratio formula (R = sample sec/ normal sec). However, in order to minimize the dependence on different reagents and instrumentation, the International Sensitivity Index, or ISI, is added to the formula as a power function. ISI is individual for each reagent and instrument pair. It stands in relation to reagent (thromboplastin) sensitivity. Being a power function ISI causes a mathematical unlinearity if it diverges from the value 1.00, its impact grows and the unlinearity of the function increases. If the equation were INR = $\left(\frac{Sample\ Sec}{Normal\ Sec}\right)^{\mathrm{ISI}}$ then the sensitivity correlation between reagents would be linear.(14)

ISI calibration using International reference preparation:

The aim in the original recommendation (WHO 1983) was to harmonise INR result by calibrating reagent ISI values with Human Combined, which was the primary reference preparation. For this purpose, their mathematical relationship was used. Thromboplastin from different sources (human brain, rabbit brain, rabbit lung and ox brain) yield quite different levels of PT. The alternative means of determining the International Sensitivity Index is the use of freshly pooled plasmas from 20 normal individuals and 60 patients on OAT. These numbers of samples are necessary to obtain a precise calibration line for ISI calculation. Freshly pooled plasma can be used to determine reagent/instrument ISI with acceptable precision, or as good as with the WHO calibration model.

PT tests in different laboratories using different thromboplastin reagents may lead to different result even when the plasma warfarin concentration is the same. This could potentially cause the misinterpretation of patient's anticoagulants status and consequently the risk of bleeding or thrombosis. To avoid this, reagents responsiveness is now determined by comparison with an internationally agreed on reference reagent: the WHO reference thromboplastin.

The WHO reference thromboplastin has an ISI of 1.00. This ISI of 1.00 corresponds to appropriate responsiveness of the PT to the effect of warfarin. As the ISI increase thereis less responsiveness to the same (plasma) concentration warfarin. For this reason laboratories should use thromboplastin with lower ISI (less than 2.00)(15)

Normal plasma calibration:

Better precision and accuracy in PT reporting are needed for the INR system are compared with the second or ration units.

One potential source of error is the mean normal Prothrombin time (MNPT). This has a major influence in the INR calculation as the denominator. A laboratory can estimate the MNPT from a minimum of 20 healthy individuals with relatively equal mix of both sexes over a range of age groups. MNPT samples must be fresh. The MNPT should be determined with each new lot of PT reagent.

D' Angelo and associates (1997) made a comparison for mean normal PT (MNPT), fresh normal pooled plasma (FNPP) and for lyophilized normal pooled plasma. MNPT and FNPP samples were obtained from the same 30 healthy subjects. Mean PT ratios did not differ from 1.00 (mean 1.00, range 1.00 0 1.01) with the use of the MNPT as denominator in the INR calculation equation. The conclusion was that the system-specific MNPT is the best alternative for normal plasma PT in calculation of INR values. (17)

1.10.3 Oral Anticoagulants :

Warfarin, a derivative of 4-hydroxycoumarin, is oral anticoagulant most commonly prescribed for venous thrombosis. Warfarin interferes with the metabolism of vitamin K. this vitamin is a required cofactor for the synthesis of coagulation factors II, VII, IX and X. warfarin has no direct effect on clot lysis. The laboratory test to monitor warfarin therapy has historically been the one-stage PT. The PT reflect the effects of warfarin on factors II, VII, X. standardization of the PT has monitor of effective oral therapy introduced in 1983 with the INR system. This occurred after recognition that they were marked different in the responsiveness of thromboplastin reagents to the vitamin K dependent clotting factor level. The thromboplastin reagents contain tissue factor phospholipid and calcium the active in grading in the reagents is the lipoprotein extract from human or animal tissue. The PT result various according particular thromboplastin reagents "responsiveness"

(sensitivity) to the effect of warfarin on factors II, VII and X. (17)

1.10.4 Lack of reliability of the INR system at the onset of warfarin therapy:

PT is responsive to warfarin-induced reduction of coagulation factors II, VII, IX and X. PT reagents vary in their sensitivities to any decrease in these factors, since their plasma half-lives are not similar. During the first two to five days from commencement of OAT, PT in INR units does not give the real picture of the situation in vivo, and in this regard the situation is similar to action % and second units. Prolongation of PT is mainly the result of a reduction in factor VII, with some contribution from a decrease in factor X levels. In long-term therapy, INR values reflect well the decrease in all three factors II, VII and X, which are dependent on K-vitamin.

Warfarin is now the most widely used drug world-wide for OAT, and it has a predictable onset and duration of action and excellent bioavailability. It is rapidly adsorbed from the gastrointestinal tract (within90 min) and its half-life is 36-42 h. In the circulation warfarin is bound to plasma proteins (mainly albumin). It accumulates rapidly in the liver, where it is metabolized. (16)

1.10.5 Single or duplicate analysis for automated PT determination:

PT measuring techniques have been developed from manual to automate during the last few decades. In smaller laboratories and world-wide, automated instruments are not necessarily available. For coagulation tests an old tradition has been to use manual methods to estimate the mean of duplicate analyses in order to confirm reliable result. This practice is still recommended for manual or partly manual techniques. Levy and colleagues (1997) studied 4152 PTs using a coagulation

analyzer and found statistical agreement between the traditional mean duplicate result and the single test. (17)

2 Rationale & Objectives:

2.1 Rationale:

Many Sudanese patients who are currently on oral anticoagulant therapy, the Prothrombin Time (PT) and/or International Normalized Ratio (INR) are the laboratory test(s) performed to monitor the medication dosage. Once the patient's PT/INR values are within the therapeutic range; the patients are usually monitored with PT/INR testing every three-six weeks. That equals more 10 million PT/INR results being reported out each year which give major source in mistakes in result according to the number of reagents/instrument used in Khartoum laboratories state this leading to effect on the dosage range of OAT for patients which may patient takes dose over than needed that leading to increase incidence of thrombosis in other side when patient taking dose less than needed may leading to acquired bleeding which seriously effect on their lives may leading to death. According to the variation of the Prothrombin reagents which have been used in Khartoum's state hematological laboratories we need to defined if there are difference between the used reagents in our laboratories and how much the quality of these reagents.

2.2 General Objective:

To assess of Measurement of Prothrombin Time using Different manufactured Prothrombin Reagents Used in Khartoum State Laboratories

2.3 Specific Objective:

- To measure clotting time for both control and patients sera using different PT reagents.

- To identify if there is a difference obtained between reagents.
- To check the accuracy of each reagent.

3 Materials and method:

3.1 Study design:

Cross sectional analytical descriptive comparative study conducted in Al-Zaiem Al- Azhari University and Khartoum Teaching hospital.

3.2 Study Approach:

This research was comparative research to assess the Prothrombin time reagents in Khartoum states laboratories.

3.3 Study population:

Different Prothrombin reagents (Stago, TEclot, futura system, fortress) which used in Khartoum state laboratories including voluntary sampling for 29 citrated blood samples (taking oral Anticoagulant therapy) obtained from Sudan heart center and Human normal control sera (Technoclot company).

3.4 Determination of Prothrombin Time:

3.4.1 Principle:

- The Prothrombin time is a screening coagulation assay that assesses the function of factor VII, factor X, factor V, Prothrombin and fibrinogen. The PT is performed as a one-step reaction first described by Quick.
- Clotting: Scattered Light Detection Method, is the principle for Sysemx CA 500 Automated method.

3.4.2 Supplies and Equipment:

- 12×75 glass test tube and rack.
- Pipettes and tips: 100µL and 200µL

- Kimwipes or gauze
- Thromboplastin reagents (Stago, TEclot, fortress and futura system)
- Timer
- Waterbath
- Centrifuge
- Reagents sheets
- Control normal (TEclot and Technoclot)
- Patient citrated plasma specimen.
- Distilled water

3.4.3 Reagents:

a. Stago Neoplastin© CI plus:

Stago is diagnostic company for Research & Development and Production to Logistics, Marketing, Sales and International Distribution, Stago remains in control of its strategy at all levels. The group's industrial activities are mostly concentrated in France. Its geographical expansion has now led to production centers in the USA, Netherlands, Germany and Ireland.

- **Reagent 1:** lyophilized thromboplastin prepared from fresh rabbit cerebral tissue which contains specific heparin inhibitor.
- **Reagent 2**: aqueous solvent containing calcium, 2ml per vial.
- **Reagent preparation and storage:** transfer the content of reagent 2 into vial of reagent 1, allow at room temperature(18-25c) for 30 mins. Storage of the reagent important in saving the stability of the reagent which remains stable for: 8 hrs at 37c – 48 hrs at 20c – 8 days at 2-8c.

b. FUTURA SYSTEM S.r.l.:

FUTURA SYSTEM is an Italian company located in Rome and operating in the diagnostic market for Clinical Laboratories since 1984. FUTURA SYSTEM s.r.l. manufactures and distributes latest generation, high quality in vitro diagnostics.

- Reagent: Rabbit brain tissue 2.6 %, Glycine 2 %, Tricine 1.34 %, Sodium solfate 0.7 %, Polyethylene glycol 0.7 %, Calcium Chloride 0.13 %, Brij 35 0.05 %, Sodium Azide 0.013 %.
- Storage and Stability of Reagents: Store the Kit at 2 - 8°C (Do not freeze) The Reagent is stable until the expiration date stated on the label

c. TEclot PT-SR liquid:

TECO was established in 1990 by a product takeover from company LRE medical technology, Munich. In the last 20 years the company grew constantly every year with the new developments and new distribution partners. The company distributes its products worldwide in more than 60 countries and additionally through well known OEM (private label) partners.
- Reagent: PT reagent contains an extract of rabbit with buffer, stabilizers and calcium chloride.
- Storage and Stability of Reagents: unopened reagents are stable until the expiration date shown on the label stored at 2-8c, opened reagent: 14 days at 2-8c – 5 days at 20c – 1 day at 37c

d. Fortress diagnostics:

Fortress Diagnostics is a rapidly expanding, innovative primary diagnostics manufacturer based in the United Kingdom, specializing in the development, manufacturing and distribution of a range of Medical Diagnostic and Point of Care Tests for a global market.
- Reagent: PT reagent is liquid ready to use rabbit thromboplastin, calcium chloride, buffer, and 0.05% sodium azide as preservative.
- Storage and Stability of Reagents: reagent is stable up until the expiry date shown on the bottle 2-8c.

e. TEControl N:

- Contents: 10×1ml freeze dried citrate-anticoagulanted human plasma.
- Preparation: reconstitute individual vials with 1ml distilled water, allow to stand at room temperature for 15mins.

f. Technoclot Control N:

- USE**:** as accuracy control of screening tests (PT/aPTT/TT/fibrinogen) within normal coagulation activity. Precision control, i.e. the recording of the variation, is an important procedure in coagulation testing; checking the accuracy of the test system is particularly important.
- Composition**:** is prepared from selected citrated plasmas of at least 100 donations of healthy donors.
- Storage and Stability of Reagents: the expiry date printed on the labels applies to storage of the unopened bottlesat 2-8c, stability after open; 4 hrs at 20c 8 hrs at 2-8c - 1 month at -20c.

3.4.4 Specimen collection and treatment:

Venous blood 9 vol is collected in trisodium citrate anticoagulant 1 vol 0.109 M (3.2%) , 4.5 ml of venous blood + 0.5 ml of trisodium citrate anticoagulant, centerifugated at 15 minutes at 2000-2500g. obtained plasma had been stored at 8 hours at 20 ^{0}c until processed.

3.4.5 Sample:

- 29 citrated blood samples (patients under oral Anticoagulant therapy) obtained from Sudan heart center hospital
- Normal control sera from Technoclot Company (lot No. 1P9DA00)

3.4.6 Procedure:

1- Thromboplastin reagents pre-warmed for 2 minutes in (37°C) before use.
2- Added 0.1 ml normal plasma and warmed to 37°C for 2 minutes
3- Added 0.2 ml pre-warmed (37°C) thromboplastin reagent. (for Automated method we add 50µL form samples and 100µL from reagents).

	Serum	Control sera
Reagent	0.2 ml	0.2 ml
Serum	0.1 ml	
Control sera		0.1 ml

4- Started stopwatch, mixed and recorded clotting times (manual).
5- Performed all tests in duplicate. Duplicate clotting times should not differ by more than 10%
6- Reported patient's clotting time in seconds

3.4.7 Calculation:

- PT = in seconds
- INR = $(\frac{Sample\ Sec}{Normal\ Sec})^{ISI}$

3.4.8 Reference Value of Control Sera:

Normal control sera (***Technoclot)*** = INR = 1

3.5 Data Analysis:

The data will be analyzed and processed by suitable statistical package of social sciences (SPSS) program by using T-test. The P-value statistical used to determine the statistical differencesif been $\succ 0.05$, that mean the difference is not significant, and if P-value $\prec 0.05$, that mean the difference is significant

4. Results:

Twenty nine venous blood samples (patients under oral anticoagulant therapy) were investigated to assess the difference between Prothrombin Reagents Company (Stago, TEclot, Futura system, Fortress). In addition, using human normal control sera to check the accuracy of used reagents. This based on comparative study carried out during the period of March 2012 – July 2012 in Alzaiem Al-Azhari University and Khartoum Teaching hospital. When we were assess the difference between the four companies using samples by analysis PT result showed descriptive statistical of results were (mean=42.68) by Stago company, (mean=44.81) by TEclot company, (mean=55.90) by Futura System and (mean=29.53) by fortress company. Showed in figure (4-1)

The P-value paired statistical for difference between PT (Stago) and PT (TEclot) the difference is not significant value between all companies showed there are significant differences in PT result. Showed in Table (4-2).

The descriptive statistical results for INR were showed (mean=3.94) by Stago, (mean=2.97) by TEclot, (mean=4.27) by Futura System and (3.15) by Fortress. Showed in figure (4-2)

The P-value paired statistical for difference showed, P-value between INR (TEclot) and INR (Fortress) difference is not significant. But the P-value between all other companies showed there are significant differences in INR results. Showed in Table (4-3).

Control sera analysis were by measuring the PT result for four companies then calculate the mean showed in Table (4-5). The normal value of INR in control sera was equal 1, when calculating the INR for the companies the P-value difference between INR results for all companies against normal control sera showed the difference is not significant expect for INR

(Futura) results the difference is significant.. Showed in Table (4-6)

According to normal value of INR, the INR mean of the control sera among the company Reagents showed the Fortress is most accurate company (mean=1), although the Stago company (mean=0.99) and TEclot company (mean=0.97) gave acceptable result according to normal value of control (0.91-1.09) but the Futura System (mean=1.16) gave statistical significant difference. Showed in figure (4-3)

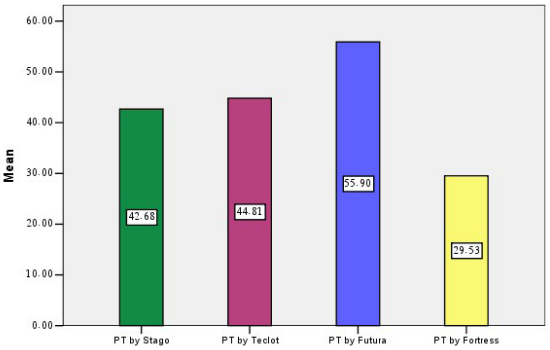


Figure (4-1). The mean of PT for patients samples using different company reagents

Table (4-2): P-value Paired statistical difference of PT result between four companies

Pair Std.	Deviation	N	Sig. (2-tailed)
PT by Stago-PT by TEclot	7.43481	29	0.135
PT by Stago- PT by Futura	9.49859	29	0.00
PT by Stago- PT by Fortress	3.85085	29	0.00
PT by TEclot- PT by Futura	9.42788	29	0.00
PT by TEclot- PT by Fortress	9.00509	29	0.00
PT by Futura- PT by Fortress	10.77894	29	0.00

Figure (4-2): The mean of INR of patients among company reagents.

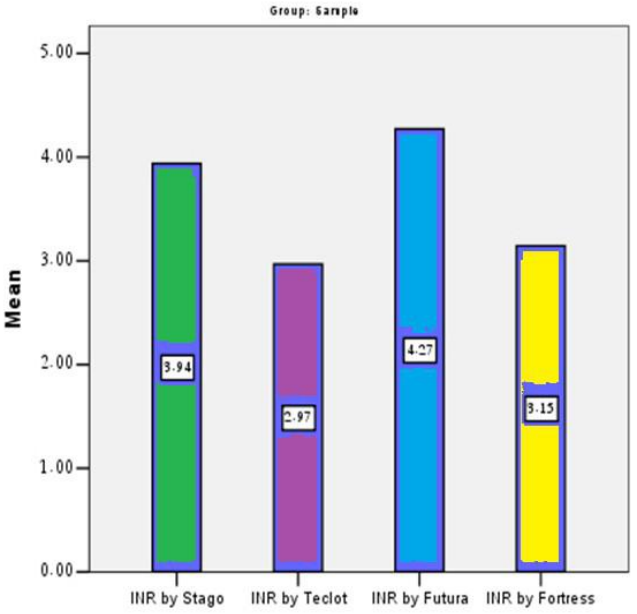


Table (4-3): P-value Paired statistical difference of INR result between four companies

Pair	Deviation	N	Sig. (2-tailed)
INR by Stago- INR by TEclot	.67990	29	0.000
INR by Stago- INR by Futura	.85736	**29**	0.046
INR by Stago- INR by Fortress	.43853	**29**	0.00
INR by TEclot- INR by Futura	.67092	**29**	0.00
INR by TEclot- INR by Fortress	.74202	**29**	0.208
INR by Futura- INR by Fortress	.91432	**29**	0.00

Table (4- 4): PT results for four companies with Technoclot control sera.

	Pt by Stago	PT by TEclot	PT by Futura	PT by Fortress
Group	Control sera	Control sera	Control sera	Control sera
Number	5	5	5	5
Mean	12.6400	10.7400	15.0200	11.9600
Std. Deviation	0.05477	0.11402	0.04472	0.11402

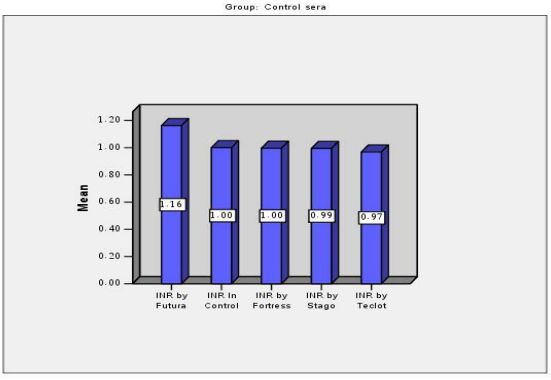
Table (4-5): INR results for four companies with Technoclot control sera.

	INR by Stago	INR by TEclot	INR by Futura	INR by Fortress
Group	Control sera	Control sera	Control sera	Control sera
Number	5	5	5	5
Mean	0.9940	0.9680	1.1620	0.9960
Std. Deviation	0.00548	0.06140	0.00447	0.01140

Table: (4-6): P-value Paired statistical difference of INR result between four companies and control sera

		N	Sig.
Pair 1	INR by Stago- INR by TEclot	5	.424
Pair 2	INR by Stago- INR by Futura	5	.000
Pair 3	INR by Stago- INR by Fortress	5	.704
Pair 4	INR by TEclot- INR by Futura	5	.002
Pair 5	INR by TEclot- INR by Fortress	5	.387
Pair 6	INR by Futura- INR by Fortress	5	0.00

Figure (4-3): The mean of the INR of the control sera among the company reagents



5. Discussion:

This study based on analytical cross sectional case-control carried out from Sudan Heart Center hospital during the period of March 2012 – July 2012, twenty nine citrated venous blood samples (patients under oral anticoagulant therapy) have been

tested to assess the difference of results between the four companies (Stago, TEclot, Fortress and Futura System).

Nancy Grove study, Kristine Rotzoll study The University of Iowa Hygienic Laboratory in 2006, the laboratory should draw patients with PT/INR results within the therapeutic range and a normal patients in order to perform comparison studies. This is done to verify the consistency of the different PT reagents. After analyzing the data, it appears that the PT/INR results are running higher or lower with the differ reagent.

Our study showed there is statistical difference between PT reagents manufactured by different companies in PT/INR results and ISI value which apparently agreed with previous studies. The results showed there are significant different in PT results between all the companies except between Stago Company and TEclot company which there no significant difference (>0.05). The INR results of these samples showed there are significant difference between all companies expect TEclot Company and Fortress.

Evaluation the quality of reagents according to their accuracy and precision by using Technoclot control normal which expressed its normal value in INR unit which equal 1.00.When we did the PT test against normal control by automated method (sysemx CA-500) five times for each companies, the results were showed there are differences between the companies ranging from 10.5-15 sec., however, when we calculate the INR to check the accuracy the result shows the best company is Fortress (INR=1), and the other companies gave no significant difference according to reference Value (0.91-1.09) expect future system company which have significant difference to control value. This difference in accuracy may return to the quality of material used, equipment, compositions or the method of storage of reagents.

Conclusion:

In this study conclusion describe as following:

- There are significant differences in PT sample results between reagents, expect between Stago and TEclot.
- All the reagents companies are accurate in results expect futura system company.
- The best reagent is Fortress, Stago, and TEclot then futura system according to ormal control. In the precision (stability) the best reagents is Futura system.

Recommendation

- More studies in other different reagents are needed to establish the quality of other reagents.
- Every regional laboratory should establish it is own check of information that present in the reagents.
- Further studies are needed to establish other control levels (abnormal) high and low control which are not included in this study.
- Further studies are needed for checking the ISI by using reference thromboplastin reagents

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