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Practice of Prothrombin Time, Activated Partial Thromboplastin Time and Mixing Test in Public Hospitals of Addis Ababa, Ethiopia, from March - June 2016

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This is to certify that the thesis prepared by Gelila Biresaw, entitled: **"Practice of Prothrombin time, activated Partial Thromboplastin Time and Mmixing Test in Public Hospitals of Addis Ababa, Ethiopia, from March - June 2016"** and submitted in partial fulfillment of the requirements for Master of Science Degree in Clinical Laboratory Science (Hematology and Immunohematology Specialty Track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
aPTT	Activated Partial Thromboplastin Time
CDC	Centers for Disease Control and Prevention
CPA	Control Plasma Abnormal
CPN	Control Plasma Normal
CSCT	Colloidal Silica Clotting Time
DRERC	Departmental Research and Ethical Review Committee
ED	Emergency Department
EQA	External Quality Assurance
EQAS	External Quality Assessment Scheme
FMHACA	Food, Medicine and Health care Administration and Control Authority
FMOH	Federal Ministry of Health
FVIII	Factor VIII
GLP	Good Laboratory Practice
HMWK	High Molecular Weight Kininogen
INR	International Normalized Ratio
IQC	Internal quality control
KCT	Kaolin Clotting Time
LA	Lupus anticoagulants
LQA	Laboratory Quality Assurance

MLS	Medical Laboratory Science
NGO	Non-government organization
OAT	Oral Anticoagulant Therapy
OPD	Out Patient Department
PK	Prekallikrein
POC	Point Of Care
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RPM	Revolution per Minute
RVV	Russell Viper Venom
SOPs	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
TASH	Tikur Anbessa Specialized Hospital
UK NEQAS	United Kingdom National External Quality Assessment Scheme
USA	United States of America
VKAs	Vitamin K Antagonists
vWF	von Willebrands Factor
WFH	World Federation of Hemophilia
WHO	World Health Organization

Abstract

Background: Coagulation tests are vital to the diagnosis, treatment as well as management of bleeding and hypercoagulability disorders. Inappropriate performance of coagulation tests, such as the prothrombin time (PT), activated partial thromboplastin time (aPTT) and mixing test contributes to coagulation and bleeding complications including death. Practice of coagulation tests is not well documented in resource limited settings like Ethiopia.

Objective: To assess the practice of PT, aPTT and Mixing test in public hospitals of Addis Ababa from March - June 2016.

Methodology: A prospective hospital based cross-sectional study was conducted from March – June 2016 in all the 13 public hospitals of Addis Ababa. Standard questionnaires and checklists were used as a tool to investigate the practice of PT, aPTT and mixing test in the respective hospitals. On site assessment was carried out using a checklist. Questionnaires were used to interview all laboratory professionals working in hemostasis section during the data collection period. Mixing tests were performed at Tikur Anbessa Specialized Hospital (TASH) on 40 plasma samples with prolonged PT/aPTT to demonstrate its usefulness and establish the technique in this premier referral and teaching hospital of the country. Data was entered and analyzed using SPSS version 21 employing descriptive statistics such as frequency and mean.

Result: Of all the 13 public hospitals in Addis Ababa, only 4 were performing either of the two coagulation tests. The performance of PT/aPTT tests was irregular among the 4 hospitals and did not comply with some SOPs. The results of this study indicate that the laboratories do not follow certain testing guidelines. None of the hospitals perform mixing test. Out of the 40 patients for whom mixing test was performed, 18 had a corrected mixing test results, suggesting factor deficiency while 22 had no correction suggesting presence of inhibitors.

Conclusion: The practice of PT/aPTT tests in the public hospitals of Addis Ababa is insufficient. Regardless of the vital role the mixing tests plays in the diagnosis, treatment and follow up of patients with coagulopathy, it is not performed in any one of the public hospitals and none of the laboratory professionals had the knowledge of it. This warrants efforts in establishing the test at least at TASH.

Key words: Coagulation, PT, aPTT, Mixing Test, Hemostasis, public hospitals

1. Introduction

1.1 Background

Coagulation profile tests such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) are screening tests for hemostasis (1, 2). Hemostasis is a complex process in which multiple components of the blood clotting system are activated in response to vessel injury, to control bleeding. Hemostasis is composed of different major events triggered through two pathways. The extrinsic pathway starts with activation of coagulation factor VII by tissue thromboplastin released from the injured tissue. Tissue factor complexed with activated factor VIIa in the presence of calcium and phospholipids activates coagulation through conversion of factor X to Xa and also through activation of factor IX to IXa, according to the alternative model (3). This pathway is evaluated with PT (1).

The aPTT measures the functional integrity of the intrinsic and common pathways of the coagulation cascade (factors XII, XI, IX, VIII, V, II, I, prekallikrein (PK) and high molecular weight kininogen (HMWK)). This pathway is initiated by the interaction of Factor XII with a negatively charged surface and being auto activated to factor XIIa. In an *in vitro* coagulation, the intrinsic pathway starts by contact activation of substances like glass, kaolin, celite, or silica to activate factor XII. In this process, HMWK and kallikrein are essential cofactors facilitating the activation. Activated factor XII subsequently activates factor XI, with factor XIa activating factor IX to factor IXa. Factor IXa, together with factor VIIIa, phospholipids, and calcium form the complex that activates factor X (2, 3).

The common pathway begins with the activation of factor X through the intrinsic pathway, the extrinsic pathway, or both. In the presence of factor V, Ca^{2+}, and phospholipids, it converts prothrombin to its active form, thrombin. The main action of thrombin is to catalyze the proteolysis of the soluble plasma protein fibrinogen to form fibrin monomers that remain soluble. Fibrin monomers then polymerize to form a gel of fibrin polymers that trap blood cells. Thrombin also activates factor XIII to XIIIa and mediates the covalent cross-linking of the fibrin polymers to form a mesh termed stable fibrin, which is less soluble than fibrin polymers. Other agents like Russell viper venom (RVV) activates factor X directly; Taipan, Textarin and Ecarin venoms are direct activators of prothrombin (factor II) (4). Thrombin activates factor V and VIII

to Va and VIIIa, respectively. Because the common pathway contains the factors X, V, and FII (any deficiency may lead to a hemorrhagic disorder), these factors may be monitored by both PT and aPTT (5).

The coagulation tests are vital to the diagnosis, treatment, and management of bleeding and hypercoagulability disorders. The tests are used as monitoring tools for patients with coagulopathy as the use of therapeutic agents tend to be based on their corrective effect on laboratory tests of hemostatic function (6). Inappropriate performance of coagulation tests, such as PT and PTT, may contribute to coagulation and bleeding complications (7).

Oral anticoagulant therapy (OAT) is used for prevention and treatment of thrombosis in patients with atrial fibrillation, venous thromboembolism and prosthetic heart valves. The number of patients on OAT is steadily increasing worldwide. Although new drugs are being introduced, vitamin K antagonists (VKAs) still predominate (8). Most clinicians rely on a battery of conventional coagulation tests conducted in a central coagulation laboratory to measure key coagulation metrics, mainly aPTT, PT, and fibrinogen levels, to assess a patient's coagulation status as well as anticoagulant therapies (9).

Unfractionated heparin therapy is usually monitored with a clot based test, aPTT. Other clot based tests such as thrombin time are infrequently used but can be useful in situations such as patients with lupus who have elevated baseline aPTT levels. Direct thrombin inhibitors are also monitored using aPTT (10). The aPTT is measured as the number of seconds it takes for the patient's plasma to form a fibrin clot after the addition of an intrinsic pathway activator, phospholipid and calcium. A prolonged aPTT can be caused by a coagulation factor deficiency or the presence of an inhibitor. In addition to aPTT, other coagulation assays utilized to detect congenital and acquired abnormalities of the intrinsic coagulation pathway and to monitor patients receiving heparin include the kaolin clotting time (KCT) and the colloidal silica clotting time (CSCT) (1, 2, 11).

The PT test is used for assessment in pre-operative detection of bleeding tendencies in risk groups and the monitoring of anticoagulant therapy. The most evidently increasing use of PT is now seen in OAT control. The test is carried out by adding thromboplastin, phospholipid and an

excess of calcium to anticoagulated plasma and measuring the clotting time. PT is the most commonly used coagulation test in routine laboratories (12).

PT and aPTT tests are routinely ordered for surgical and other patients admitted to hospital even though studies consistently demonstrate the low diagnostic utility of this practice (1). The International Normalized Ratio (INR) is a standardized method for reporting results of the PT assay. Reliable determination of the INR is mandatory for the control of oral anticoagulant therapy. Determination of the INR is based on a calibration model adopted by the world health organization (WHO) (13). It is sensitive to prothrombin, tissue factor, factors V, VII and X. As these coagulation factors depend on vitamin K for synthesis, the INR reflects anticoagulation owing to warfarin. For a warfarin-anticoagulated patient, the INR can aid decision making around the measures that may be needed to normalize coagulation activity and stop or stabilize bleeding. Elevated INRs can arise from direct overdosing of anticoagulants, as well as interactions between warfarin and numerous other drugs or herbal remedies. It can also be a result of fluctuations in dietary vitamin K owing to dietary changes or intake of nutritional supplements, or as a consequence of alcohol abuse (14).

Concurrent conditions, such as hepatic or biliary disease, gastrointestinal illness or sepsis can also increase the INR. In such situations, the cause of the elevation needs to be identified so that repeated incidents and the resultant increased bleeding risk can be avoided. In the event of a self-reported and/or unexplained elevation in INR, retesting in the emergency department (ED) can confirm the elevation and exclude erroneous results. However, in the ED itself, testing artifacts such as under filling tubes when drawing blood for INR testing can yield falsely elevated INR results owing to excess levels of citrate in the test sample. Errors can be avoided by use of the correct sample tubes and ensuring that they are adequately filled. Patients are treated with warfarin to manage thrombosis or reduce the risk of stroke or other thromboembolic events. Therefore, when a VKA treated patient presents at the ED, the INR should be confirmed to be in the reference range, and intervention may be required when it is not. If necessary, the patient should be referred for appropriate warfarin management on discharge (14).

A Prolonged aPTT and/or PT, has to be further investigated by a mixing test to find out the causes for the abnormality. Mixing test is a simple procedure used in the hemostasis laboratory as a first line investigation into the cause of an abnormal screening test. The mixing test involves

combining an equal volume of the patient's plasma with normal pooled plasma, then repeating the screening test on the mixture to assess whether the test becomes normal or remains prolonged (15, 16).

It is expected that the normal plasma will correct coagulation deficiencies and normalize the PT and/or aPTT. However, if the cause for the prolonged PT and/or PTT is the presence of an inhibitor the clotting time will remain prolonged or in rare cases even show a further prolongation up on mixing (the lupus co factor effect). Most laboratories use a ratio of patient and normal plasma of 1:1 but four parts control to one part patient plasma is also used (4). Unfortunately there are no uniform criteria or precise guidelines for the interpretation of results with mixing studies until they are well defined in updated guidelines. In this area further standardization is warranted (4, 17). Mixing studies cannot be performed when the PT or aPTT is within 2 or 5 seconds of normal range, respectively (4).

Many pre-analytical, analytical and post analytical variables affect the accuracy of coagulation test results. The phlebotomy technique is the most important variable. A clean venipuncture with minimal stasis should be carried out. The presence of interferences like clot, hemolysis, lipemia or icterus should be noted as these may affect the analytical procedures and final result outputs. The use of small bore needles (< 23 gauge), entry into small veins and increased force in drawing back the syringe plunger may result in lysis of red blood cell (RBC) and the release of phospholipids that may initiate the coagulation process. On the other hand, avoiding such errors along with the use of plastic or siliconised glass collection tubes by ensuring correct filling enhances the quality of the tests (18, 19).

The effects of poorly phlebotomized blood may not become apparent until the sample reaches the laboratory (clotted sample) or during the analytical phase (decreased clotting time) or post analytical phase, when samples are saved for future testing. Blood is activated by exposure to negatively charged surfaces such as glass or plastic and delays of greater than 60 seconds in transferring freshly collected blood into vacutainer tubes containing appropriate anticoagulants may also affect test results. Other problems include tourniquet time, improper blood to anticoagulant ratio, improper anticoagulant uses, improper clearing of indwelling catheters prior to blood collection for testing, improper storage of sample once collected and delays in getting samples to the laboratory (20).

Use of sodium citrate in the concentration of 3.2 % and in the proportion of 9 volumes blood to 1 volume anticoagulant is recommended for blood sample collection. Blood samples have to be transported rapidly (ideally within 1 hour, avoiding prolonged storage at 4^0C) at room temperature and analyzed within 4 hours. As soon as possible after collection, the specimen should be centrifuged at about 1500 revolution per minute (RPM) for 15 minutes (10, 21). All in all, the coagulation tests result can be affected by variations among testing devices, reagents, patient physiology, pathophysiology, concurrent medications, improper blood collection, plasma preparation and delay in centrifuging or testing samples (22).

Knowledge about the practice of aPTT, PT/INR, and mixing tests is useful to design appropriate strategies to prevent errors that affect these tests.

1.2 Statement of the problem

Coagulation screening tests such as PT and aPTT may provide useful information particularly when full clinical details are not available which can help to avoid problems in the interpretation of coagulation assay results like anticoagulant effects or the presence of coagulopathy. PT/INR and aPTT are very common coagulation laboratory tests that are vulnerable to errors and adverse patient outcomes. Many tests used to assess hemostasis can be influenced by medication and this must be taken into account (23). PT/INR and aPTT are among the laboratory investigations performed for hemophilia, which is the most common inherited bleeding disorder (IBD) (24).

Patients on OAT must be monitored carefully to prevent dangerous complications of bleeding or thrombosis (25). High quality anticoagulation management is necessary to keep the narrow therapeutic index medications as effective and safe as possible but medical mistakes and errors are unacceptably high, despite a longstanding focus on activities carried out in the name of quality assurance, quality improvement, total quality management, and quality assessment (26).

OAT should be managed in a systematic and coordinated fashion, incorporating patient education, systematic INR testing, tracking, follow up, and good patient communication of results and dosing decisions. PT is fundamental to prevent bleeding complications or thrombotic events during oral anticoagulation therapy. Incorrect coagulation laboratory testing practices and especially those used in monitoring of patients to prevent bleeding or thrombotic events may lead to adverse clinical outcomes. Incorrect aPTT values may result in a risk of subsequent thrombosis, bleeding, overall morbidity as well as death (27).

To improve the safety of patients undergoing coagulation laboratory tests, it is necessary first to assess the performance coverage and the extent to which those hospital laboratories performing the test adhere to the accepted testing practices since variations in such practices could have a direct impact on clinical outcomes (28, 29).

Mixing studies are very simple tests used to determine whether a prolonged PT or aPTT is due to a factor deficiency or the presence of an inhibitor. Mixing studies are based on two principles of which the first is the presence of any inhibitor in excess will inhibit normal and patient plasma, and the second is that 50% of any factor is enough to yield a normal test result. Correction into

the reference interval indicates a factor deficiency. Lack of correction suggests the presence of an inhibitor (30).

The primary purpose of mixing test is to guide further investigations. When mixing test results normalize, this suggests the test plasma is deficient in clotting factor(s). When the mixing test result does not normalize, this suggests the presence of an inhibitor or other type of interference (31).

Moreover, little is known about the various testing practices in hospital laboratories. In response to the uncertainty surrounding coagulation testing practices, this study was conducted. All governmental hospitals were selected because the tests are included in the test menu of their respective laboratories as per the standards of the Food, Medicine and Health care Administration and Control Authority (FMHACA) of Ethiopia.

Therefore the current study was set to determine the practice of these tests in public hospitals of Addis Ababa, which is fundamental to identify the existing gaps and properly guide the necessary intervention actions. In addition, mixing test was performed on 40 patient samples with prolonged PT and aPTT test results at Tikur Anbessa Specialized Hospital (TASH) to demonstrate its usefulness and establish the technique in this largest national referral and teaching hospital which also has the handful hematologists of the country.

1.3 Rationale

The aim of this research was to reveal the actual performance of PT, aPTT and mixing tests in all public hospitals of Addis Ababa in order to fill the gaps that exist in hematology (coagulation) laboratories. It tried to contribute to a better case management of patients with coagulopathies and bleeding disorders.

The finding will help to prevent, if not reduce, the current trend in the health care provision of our country, which is sending of samples with prolonged PT/aPTT results out mostly to Germany or South Africa through private laboratories in order to have the follow up investigative tests done. This practice is resource intensive in terms of cost and time. The cost per test in order to have the tests done abroad is up to 500 Ethiopian birr with a minimum 1 week turn over time to receive the test results. Mixing test is a simple and affordable test procedure which helps guide the factor assay tests and inhibitor identification tests on samples with a prolonged PT/aPTT test results. By demonstrating the importance, cost effectiveness, ease in performance and time effectiveness the test has, this study plays a significant role in introducing the mixing test to the laboratories of our public hospitals.

Through this research the degree of adhesion to standard operating procedures (SOPs) in the determination of PT and aPTT tests were evaluated and gaps identified. The pre-analytical, analytical and post-analytical quality aspects of the test's performance were determined. As a result, the study greatly contributes to the quality performance of PT/aPTT as well as to the introduction of the mixing tests into the testing systems of our hospital laboratories. This ultimately moves forward the clinical management of patients with hemostatic abnormalities.

In conducting this research, we gained a better understanding of the state of coagulation testing and the extent of its variability among the public hospitals of Addis Ababa. The current research plays a significant role in increasing awareness of preventable medical errors, leading to efforts to create systems that will help detect and eliminate them as easy and fast as possible as the coagulation laboratory testing is an area that has a great potential impact on patient safety and yet very much neglected in our country.

2. Literature review

Earlier reports in the 1980's indicated that coagulation tests like aPTT, mixing tests, qualitative factor assays, and bleeding times were part of the routine coagulation protocols (32). With the advent of modern instrumentation and associated improvements in test performance and reliability as well as the introduction of appropriate internal quality control (IQC) and external quality assurance (EQA) measures, substantial developments occurred to reduce analytical errors within hemostasis (coagulation) laboratories (33). However, incorrect or inappropriate test results still occur, perhaps even as frequently as in the past, primarily in the pre-analytical phase. In addition, errors related to sample collection and processing, post-analytical errors related to the reporting and interpretation of test results are also part of the challenges (33).

In recent years, there has been a marked increase in the workload and scope of the coagulation laboratory and this has been accompanied by the introduction of increasingly complicated and automated equipment employing a variety of different techniques. It is apparent that the potential for error is considerable and for this reason it is essential that the performance of laboratories be monitored through its participation in an EQA programme (34).

Practice standards and guidelines are developed through a consensus process that identifies specific essential requirements for materials, methods, and practices. They are designed to both establish and harmonize best practices among the health care community. However, studies have shown that despite required and voluntary standards of practice, many laboratory professionals fail to use them. A report in 1990 revealed the magnitude of medical errors and concluded that most were the result of systematic failures and were preventable (35, 36).

Mounting body of evidence is available regarding the significance of the common coagulation tests like PT/INR and aPTT as well as factors affecting them (37-40). For example, Paul Froom and his collaborators evaluated the reliability of delayed PT/INR determinations in a central laboratory using offsite blood sampling in Israel. The result, which was published in 2001, revealed that the INR of a centrifuged and uncentrifuged blood sample left at room temperature for 24 hours consistently increased by 6%. After adjustment of the increment, there were no misclassifications in the assessment of the adequacy of anticoagulant treatment. The researchers also compared the INR differences in the blood of patients receiving oral anticoagulants after 24

hour storage in four conditions: centrifuged at room temperature, centrifuged at 4°C, uncentrifuged at room temperature and uncentrifuged at 4°C (39).

Inconsistent changes were noted in tests of refrigerated centrifuged blood. The researchers concluded that storage of blood at room temperature for 24 hours results in a consistent prolongation of the PT, which after correction (multiplication by 0.94 for blood stored at room temperature for 24 hours, either with or without centrifugation) can reliably be used to adjust the dose of oral anticoagulants (39).

In Germany, Matthes *et al* evaluated the effect of 8 and 24 hours storage of whole blood at ambient temperature on common coagulation parameters. According to the study procedure used in this study, aliquots were taken from citrated whole blood of inpatients and outpatients (n=147) within 4 hours after blood withdrawal and after extended storage of whole blood for 8 hours and 24 hours at ambient temperature. After centrifugation, aliquots were analyzed for PT, aPTT, fibrinogen, antithrombin, thrombin time and D-dimer. The findings show that the mean percentage change after 8 and 24 hours storage was below 10% for all parameters and that all parameters can be reliably tested after 8 hours storage considering changes in individual samples. The acceptable storage time can be extended to 24 hours for PT, thrombin time and D-dimer. Clinically relevant changes were detected after 24 hour storage for aPTT. After 24 hours storage, changes for fibrinogen and antithrombin values were clinically acceptable except for borderline samples (40).

Studies assessing the practice of PT/INR, aPTT and mixing tests are limited. Evidences are generated from external quality assessment scheme (EQAS) participation. While regular participation in EQAS is critical for ensuring acceptable laboratory performance, participation in such programs is uncommon for laboratories performing tests of hemostasis in developing countries. There are several reasons attributing to this cause, including the lack of awareness of its significance, absence of locally administered and easily accessible programs and costs associated with some of the international schemes. To address this problem, experience from India demonstrated that establishment of an EQAS for hemostasis improved the practice of hemostatic laboratories. For example, as reported by Mammen *et al* in 2007, 60 to 95% of laboratories in India had their clotting times and 57 to 77% of them had their factor assays within consensus. The program has helped identify causes of unacceptable performance (41).

In 2009, Jennings *et al* reported the performance of laboratories as part of the World Federation of Hemophilia (WFH) EQA program during the period 2004-2007. Samples for PT, aPTT, factor VIII:C, factor IX:C and VWF assays were distributed to centers in both established and emerging countries, and results were compared with results obtained by United Kingdom National External Quality Assessment Scheme (UK NEQAS) participants on the same samples (42).

In general, good agreement was seen throughout between WFH and UK NEQAS for screening tests. Agreement between emerging and established WFH centers was comparable for screening tests, possibly indicative of the relative simplicity of these tests and the availability of automation in almost all hemostasis laboratories. Distribution of a questionnaire revealed methodological variations which may contribute to observed difference in performance. Several centers participated in supplementary exercises, with comparable results obtained by emerging and established centers performing factor VIII and fibrinogen measurement on cryoprecipitate, and all centers performing factor VIII inhibitor assays correctly identifying the presence of an inhibitor. The study concluded that participation in EQA programs should continue to encourage improvement in laboratory performance and therefore improvements in the diagnosis and care of patients with hemophilia (42).

Moffat *et al* analyzed the compliance of laboratories' performance with published recommendations for lupus anticoagulant (LA) testing. Two questionnaires were distributed to clinical laboratory members of the North American Specialized Coagulation Laboratory Association (NASCOLA) and the ECAT Foundation (ECAT). The result showed that the commonly performed LA tests included the dilute Russell's viper venom time, LA sensitive aPTT and hexagonal phospholipid test. The majority complied with published recommendations: to use platelet poor plasma for LA tests; to use two or more screening tests, representing different assay principles, and one assay having a low phospholipid concentration to exclude LA. Other complied recommendations include, confirming LA phospholipid dependency by the method giving an abnormal LA screen; documenting the inhibitor activity on pooled normal plasma; and not using phospholipid antibodies to confirm LA. In this study, a minority (<35%) followed the recommendations to exclude factor deficiencies and factor inhibitors as the cause of an abnormal LA test (43).

Most participants of this study (79%) performed mixing studies to investigate the possibility of factor deficiencies. After participating, 32% of laboratories had changed practices and 20% indicated that they would be changing practices. While most laboratories generally follow published guidelines for LA testing, few follow recommendations to evaluate for other coagulation abnormalities (43).

In October 2003, the Washington State Office of Laboratory Quality Assurance (LQA) and the Centers for Disease Control and Prevention (CDC) created a model to collect and monitor laboratory quality indicators from a broad spectrum of clinical laboratories. Adherence to the standards they studied was relatively high for sites performing traditional PT methods. About 92% used 3.2% sodium citrate, and 94% had a written specimen acceptance/rejection policy. The majority (95%) of these sites verified their reference range (92%), and established their mean of normal for new lots of thromboplastin reagents. For sites using point of care (POC) devices, specimens were primarily obtained by capillary collection methods. Therefore, issues related to collection tubes, sample transport, processing, and storage were not applicable (25).

The study also revealed that respondents using POC devices relied more on information provided by the manufacturer for reference ranges and mean of normal values, rather than establish their own. Nearly all testing sites reported INR values. As part of this study, laboratory personnel's use of voluntary practice standards to develop their PT testing policies and procedures was determined. The finding identified that a minority of respondents used voluntary practice standards, and that the most common reason given for not using standards was a ~~"l~~ack of awareness" (25).

Shahangian *et al* conducted a survey to evaluate practices reported by hospital coagulation laboratories in the United States. The authors examined large and small hospitals as separate groups to determine whether they had different practice profiles regarding coagulation laboratory testing practices. According to their finding, 97.1% of respondents reported performing some coagulation laboratory tests. Of these, 71.6% reported using 3.2% sodium citrate as the specimen anticoagulant to determine prothrombin time (28).

Of the same respondents in this study, 45.3% reported selecting thromboplastins insensitive to heparin in the therapeutic range when measuring prothrombin time and 58.8% reported having a

therapeutic range for heparin. An estimated 96.3% of respondents assayed specimens for activated partial thromboplastin time within 4 hours after phlebotomy, and 89.4% of respondents centrifuged specimens within 1 hour of collection. An estimated 12.1% reported monitoring low molecular weight heparin therapy, and to do so, 79% used an assay for activated partial thromboplastin time whereas 38% used an anti-factor Xa assay. According to the findings substantial variability in certain laboratory practices was evident. Where significant differences existed between the hospital groups, usually large hospitals adhered to accepted practice guidelines to a greater extent. Some reported practices are not consistent with current recommendations, showing a need to understand the reasons for noncompliance so that better adherence to accepted standards of laboratory practice can be promoted (28).

In Africa, the practice of coagulation laboratories is less investigated. The few published reports that exist focus on the clinical utility of the tests (44, 45) and another one on factors affecting the tests (46). For instance, a research conducted by Adaeze *et al* in University of Calabar, Nigeria evaluated PT and aPTT of hypertensive patients attending a tertiary hospital in Calabar, Nigeria. They reported that Systolic blood pressure and diastolic blood pressure correlated positively with aPTT in hypertensive patients and that PT and aPTT were significantly higher in hypertensive patients when compared to normotensive participants. The results obtained in this study indicate that measurements of PT and aPTT may serve as indices for evaluating hemostatic abnormalities in hypertensive patients and guide for antihypertensive therapy (45).

Ndakotsu *et al* from the same country, on the other hand, analyzed the effect of plasma storage on PT and aPTT at a Nigerian public laboratory. The tests were run on plasma from 40 healthy adults using a semi-automated coagulometer. PT and aPTT were measured at 0 (within an hour of sample collection), 4, 6 and 24 hours on samples stored at room temperature, refrigerated samples and frozen samples. The values at 0 hour were compared with the values at 4, 6, and 24 hours of each storage temperature (49). One aliquot was kept at room temperature (20°–29°C); one batch of three aliquots was refrigerated (2°–4°C) and another batch of three aliquots was kept frozen (−10° to −14°C). PT and aPTT values were within the reference ranges at 0 hour. For refrigerated plasma, PT values at 4 hours were within normal, but at 6 and 24 hour, they were significantly deranged. PT was significantly different at 4, 6, and 24 hours for both room temperature and frozen plasma. The authors concluded that for reliable PT and aPTT results,

samples should be processed and run immediately after collection. However, plasma for PT can be stored at 2°–4°C for only 4 hours (46).

In Ethiopia, the status of hemostasis laboratories is at its infancy despite the clinical significance of the common coagulation tests. As there are no published data except personal experiences regarding the practice of PT/INR, aPTT and mixing tests in the country, this study was planned to generate evidences that are critical to improve the diagnosis and care of patients with coagulopathies and bleeding disorders.

3. Objectives

3.1 General objective

- To assess the practice of PT/INR, aPTT and Mixing tests in public hospitals of Addis Ababa, from March to June 2016.

3.2 Specific objective

- To evaluate the pre-analytical quality aspect of PT/INR and aPTT determination in public hospitals of Addis Ababa.
- To describe the analytical quality aspect of PT/INR and aPTT determination in public hospitals of Addis Ababa.
- To evaluate the post-analytical quality aspect of PT/INR and aPTT determination in public hospitals of Addis Ababa.
- To establish mixing test in Tikur Anbessa Specialized Hospital.

3.3 Hypothesis

The status of PT, aPTT and mixing test practices in public hospitals of Addis Ababa does comply with SOPs.

4. Materials and methods

4.1 Study area

The practice of PT, aPTT and mixing tests was evaluated using questionnaires and checklists in all public hospitals of Addis Ababa, Ethiopia. Administratively Addis Ababa is divided into 10 sub-cities and 99 Kebeles (smallest unit in the government administrative structures). As per the information gathered from Addis Ababa Health Bureau, there are more than 800 health facilities in the City of which 50 are governmental and private Hospitals. About 50 are governmental health centers and more than 700 are private clinics. Out of the 50 governmental and private hospitals, 37 are privately owned while the 13 are owned by the government.

Four of the governmental hospitals are managed under the Federal Ministry of Health (FMOH), one under Addis Ababa University (AAU) while the rest are managed under Addis Ababa City Health Bureau and two are military hospitals. The current study focused only in the 13 public hospitals of Addis Ababa, namely, Alert hospital, Armed forces referral and teaching hospital, Emanuel hospital, Federal police hospital, Ghandi memorial hospital, Menilik II hospital, Ras Desta hospital, St. Paul's hospital, St. Peter hospital, Tikur Anbessa Specialized hospital (TASH), Trunesh Bejing hospital, Yekatit 12 hospital and Zewditu memorial hospital. An average of 5 PT/aPTT tests per day and 1800 PT/aPTT tests per year are performed in the above mentioned hospitals. All the public hospitals were included in this study for assessing the practice of coagulation tests as they all are expected to perform PT/aPTT tests as per FMHACA standards.

Mixing tests were performed in Tikur Anbessa Specialized Hospital. TASH, located in the nation's capital Addis Ababa, is Ethiopia's largest referral public hospital. In 1998, TASH was given to Addis Ababa University (AAU) by the FMOH to serve the faculty as a main teaching hospital. The hospital provides a tertiary level referral service and is open 24 hours for emergency services. TASH offers diagnosis and treatment for approximately 370,000- 400,000 patients a year in its specialty clinics including hematology, renal, cardiac and gastroenterology units. The hospital has 800 beds, with 130 specialists, 50 non-teaching doctors, more than 350 residents. The emergency department cares for around 80,000 patients annually. This hospital was selected for the mixing study as it is currently the only referral center for coagulation and

other blood disorders serving patients coming from all corners of the country. The hematology unit is staffed with four hematologists providing the highest level referral service for the entire nation of more than 90 million people.

4.2 Study design and period

A prospective hospital based cross sectional study was conducted from March to June 2016.

4.3 Population

4.3.1 Source population

- All hospitals in Addis Ababa were the source population for the evaluation of practice of PT, aPTT and mixing tests by using questionnaires and checklists.
- All patients who came to TASH to attend the laboratory department's service during the data collection period for PT/aPTT testing were the source population for plasma sample collection to perform the mixing tests.

4.3.2 Study population

- All public hospitals of Addis Ababa were the study population for the evaluation of practice of PT, aPTT and mixing tests by using questionnaires and checklists.
- All patients who came to TASH during the data collection period and who had a test request for PT/aPTT with a prolonged result were the source population for plasma sample collection to perform the mixing tests.

4.4 Eligibility criteria

4.4.1 Inclusion criteria

- For the assessment of the practice of PT, aPTT and Mixing test, all volunteering public hospitals of Addis Ababa were included.
- For the performance of mixing tests at TASH, those patients who had a prolonged PT/aPTT test results and who volunteered to take part in the study were included.

4.4.2 Exclusion criteria

- Patients who were on known anticoagulant therapy.
- Children below the age of 18.
- Public hospitals which were not performing either PT, aPTT or mixing tests during the data collection period of the study were not included in the assessment of the PT, aPTT and Mixing test practice using questionnaires and checklists.

4.5 Study variables

4.5.1 Dependent variables

- Practice of PT/INR , aPTT and mixing tests
- Mixing test results at TASH

4.5.2 Independent variables

- Presence of qualified laboratory professionals
- Adherence to SOPs

4.6 Sample size and sampling method

4.6.1 Sample size calculation

All public hospitals of Addis Ababa were included for the onsite evaluation of PT/INR, aPTT and mixing test performance using standard questionnaire and checklist. A total of purposefully selected 40 plasma samples with prolonged PT/aPTT test results were analyzed for mixing tests at TASH.

4.6.2 Sampling method

Convenient sampling was implemented while conducting this research. All public hospitals of Addis Ababa were included in the assessment of the practice of PT, aPTT and mixing tests. All patients who came to Tikur Anbessa Specialized Hospital during the study period and fulfill the inclusion and exclusion criteria were included in the mixing test studies.

4.7 Data collection procedure

The data regarding the assessment of PT/aPTT performance was collected using standard questionnaires and checklists (see annex). The questionnaire was used to evaluate the performance of the PT/aPTT tests and to gather the knowledge, attitude and practice related data of the laboratory technologists in all public hospital laboratories of Addis Ababa. The checklist was used to perform an onsite assessment of all the hospital laboratories. PT, aPTT and mixing studies were performed following SOPs (see annex).

The plasma sample of patients who came to TASH during the study period fulfilling the inclusion and exclusion criteria for the mixing study were analyzed using HumaClot Duo coagulation analyzer (Wiesbaden, Germany). The results were recorded to demonstrate the ease and effectiveness of the mixing tests. The laboratory professionals' were interviewed regarding their practice in performing PT, aPTT and mixing tests. The questionnaire was delivered to each public hospital in Addis Ababa. Laboratory professionals working in the respective hospital laboratories were asked to collaborate and provide their feedback. The data involving the onsite assessment was collected using a checklist. The patients' basic information (like age and sex), PT, aPTT and mixing test results from the HumaClot Duo coagulation analyzer were recorded.

4.7.1 Sample collection and processing

As part of the patients' clinical management, whole blood was collected into 2.7 ml, 3.2% Sodium Citrate vacutainer tubes. The collected samples were centrifuged at 1500 RPM for 15 minutes to separate the plasma. In brief, to determine PT/INR 100 µL plasma was mixed with Calcium thromboplastin reagent after warming the plasma and the reagents at 37 ^{o}C. The time at which plasma is clotted was the PT which is reported in seconds as well as INR. The aPTT was determined by incubating platelet poor plasma at 37 ^{o}C; then phospholipid (cephalin) and a contact activator were added followed by adding an optimum amount of calcium chloride. All reagents and samples were pre warmed at 37 ^{0}C. Addition of calcium initiates clotting and timing begins from addition of calcium to the formation of fibrin clot. The time at which plasma is clotted was the aPTT which is reported in seconds.

The mixing test was performed for those patients having prolonged PT and aPTT results by mixing equal amount of patient and pooled human plasma with normal PT and aPTT values. After this the PT and aPTT were performed on the 1:1 mixed samples as described above. Corrected PT/aPTT test results indicated a deficiency of clotting factors while lack of correction after performing the mixing studies indicated the presence of inhibitors. The samples were analyzed on HumaClot Duo Coagulation analyzer by following standard operating procedure (SOP).

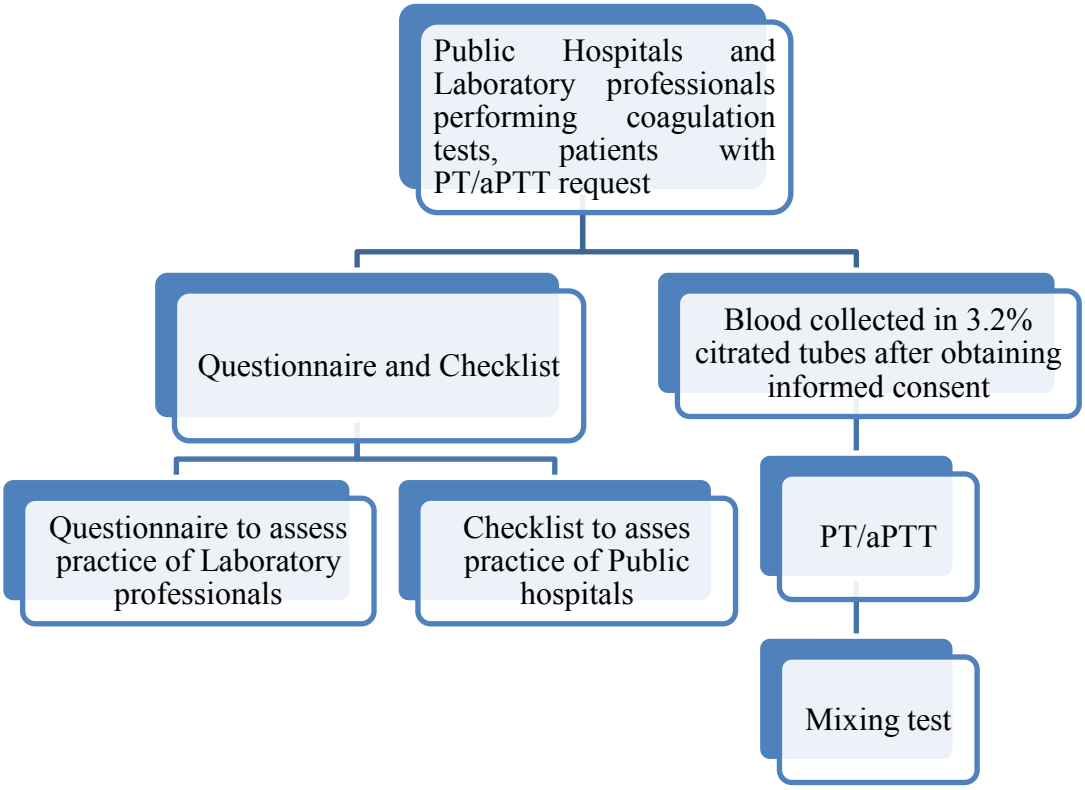

Figure 1. Workflow

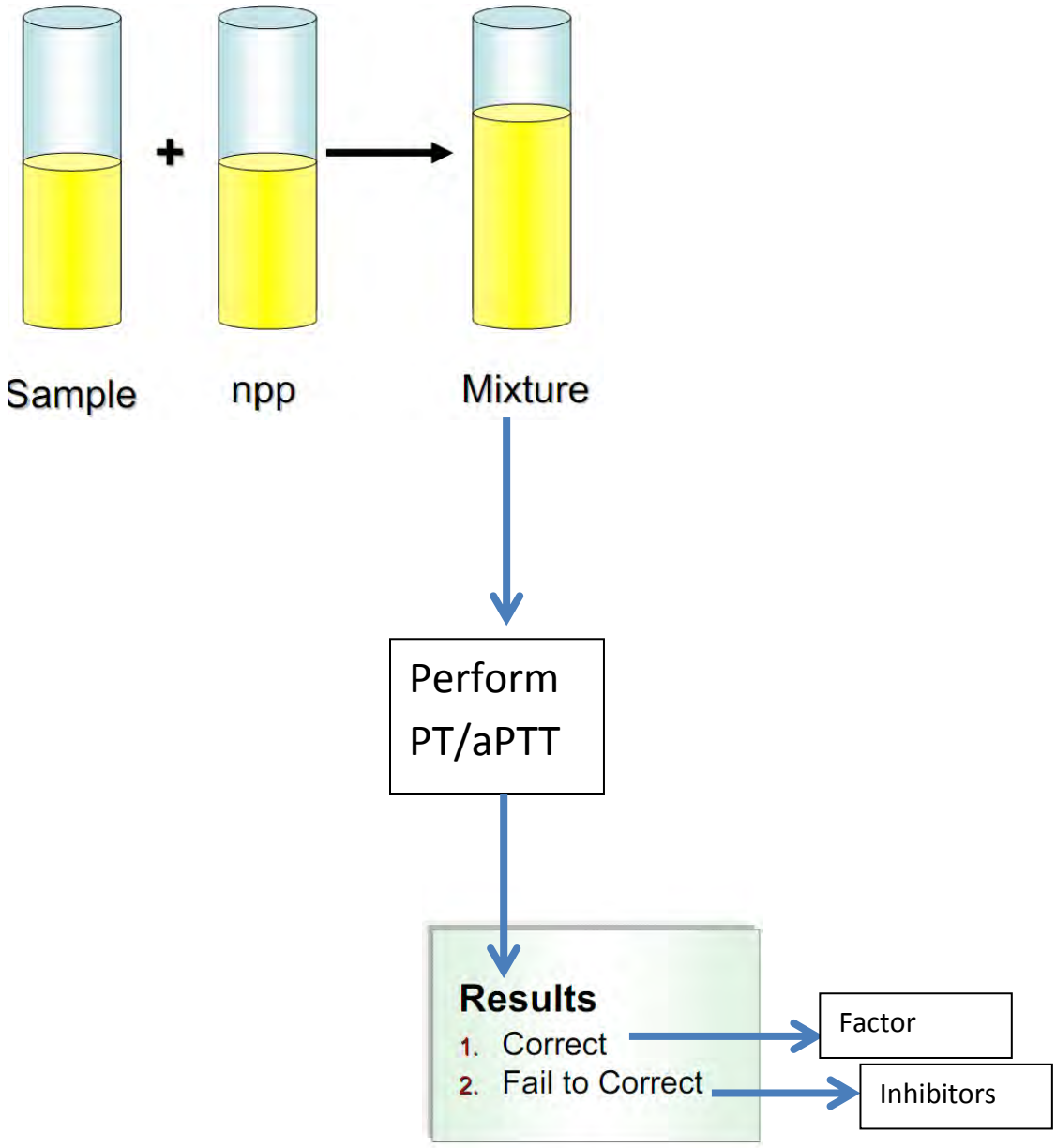

Figure 2. Mixing Test concept

Key

npp – normal pooled plasma

Sample – plasma sample

4.8 Quality Assurance

The questionnaires and the checklists were pretested in one voluntary hospital which was not included in the study and proper adjustments were made accordingly. Daily check was made on all questionnaires for incompleteness, errors and logical inconsistency at the end of each day. Prompt feedback was given on the spot during the data collection process. The methods employed were all to ensure the quality of the collected data. All aspects of quality were thoroughly controlled. Laboratory professionals involved in the study process were fully briefed specific to this study. Advisors were supervising and guiding every process in order to make sure quality of the study is maintained in each step. Samples were only being tested when daily controls lie within the acceptable range. The laboratory SOPs were strictly followed at all times. The principal investigator was involved in every step of the process including disseminating questionnaires, interviewing study participants, analyzing patients' plasma samples and running controls on the HumaClot Duo before analyzing patients' sample.

4.9 Data analysis and interpretation

The data was entered into Excel spread sheet and imported for analysis to SPSS Version 21 software (SPSS INC, Chicago, IL, USA). Data analysis was performed using scores. Descriptive statistical calculation was applied. The continuous variables were presented as the means and standard deviations. Frequency was analyzed with 95 % confidence interval to assess the practice of PT, aPTT and mixing test determination. Percentages, tables and graphs were used for depiction of the data.

4.10 Ethical considerations

The study protocol was reviewed and ethically approved by the department of Medical Laboratory Science Research and Ethical review committee, School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. A letter for cooperation was submitted to the respective 13 hospitals. Data on the practice of PT/aPTT and mixing tests determination at TASH was carried out after informing the respective laboratories about the significance of this research and the contribution the findings have in moving forward the clinical care of patients with coagulopathies and bleeding disorders. Thus, volunteering laboratories, laboratory professionals and patients with prolonged PT/aPTT test results were included in the study purely on voluntary basis. Consent was obtained from all of them for both the assessment using the

questionnaire and the checklist as well as for collecting plasma samples for the mixing study from patients at TASH.

4.11 Dissemination of result

The result of the study will be presented to the department of Medical Laboratory Science. After the defense, a hard copy of the study will be delivered to ministry of health, Addis Ababa health bureau and all the public hospitals of Addis Ababa which participated in the study so the appropriate follow up actions implementing the recommendations of the research can be taken. A copy of the research will be kept in the library so it can serve as a reference for future studies and all the readers. The study will also be presented to the scientific community in different national, regional as well as international scientific conferences. The findings will also be published in peer reviewed journals as means of disseminating the results to a larger audience. In addition, continuous dissemination will be done through oral or poster presentation of the study outputs in relevant workshops, symposiums and conferences.

4.12 Operational definition

- **Hemostasis laboratory**: A laboratory where coagulation tests like PT/INR, aPTT and mixing tests are performed.
- **Hematology laboratory:** A laboratory where hematological tests like complete blood cell count, blood cell morphology examination and coagulation tests are performed.
- **Prolonged PT/INR, aPTT:** An abnormal value outside of the reference interval for PT/INR and aPTT.
- **INR:** International normalized ratio obtained from automated coagulometers used to standardize PT values internationally.
- **Mixing test:** A test performed on a sample having a prolonged PT/INR and/or aPTT to identify the cause of the prolonged result.
- **Pooled human plasma:** plasma collected from volunteers having normal PT/aPTT.

5. Results

The study assessed all governmental hospitals namely, Alert hospital, Armed forces referral and teaching hospital, Emanuel hospital, Federal Police hospital, Ghandi memorial hospital, Menilik II hospital, Ras Desta hospital, St. Paul's hospital, St. Peter hospital, Tikur Anbessa Specialized Hospital, Tirunesh Beijing hospital, Yekatit 12 hospital and Zewditu memorial hospital. According to the national health facility standards developed by FMHACA, all laboratories of public hospitals in Addis Ababa are expected to perform coagulation tests. Unlike the hospitals, health centers are not expected to perform the coagulation tests.

5.1 Performance coverage of coagulation tests among public hospitals of Addis Ababa

Among the above mentioned 13 public hospitals of Addis Ababa, only 4 were found performing the coagulation laboratory tests. This makes the total proportion coverage of the coagulation tests performance to be 30.8% (4/13). Among the 4 hospitals found performing the coagulation laboratory tests, only 2 hospitals, perform both PT and aPTT whereas the remaining two perform PT test only. The two hospitals performing both PT and aPTT assays are the only two INR reporting hospitals among all of the four hospitals performing PT. The laboratory professionals working in the hemostasis section of the two aPTT non-performing hospitals reported that the lack of reagent availability as the reason why they do not perform aPTT test.

5.2 Result of assessment using questionnaires

5.2.1 Study respondents

A total of 20 laboratory professionals working in coagulation/hematology section of the four hospital laboratories voluntarily responded to the questionnaires prepared to assess the performance of PT, aPTT and mixing tests. Three of the 20 participants (15%) were female laboratory professionals. The participating laboratory professionals were in different academic levels. Five (25%) of them had a diploma, 13/20 (65%) had BSc degree and the rest 2/20 (10%) had MSc degree (Figure 3). The mean age distribution of the laboratory professionals who took part in this study was 20.5 years old. The minimum and the maximum age distributions of the study participants were 23 and 42 years old, respectively.

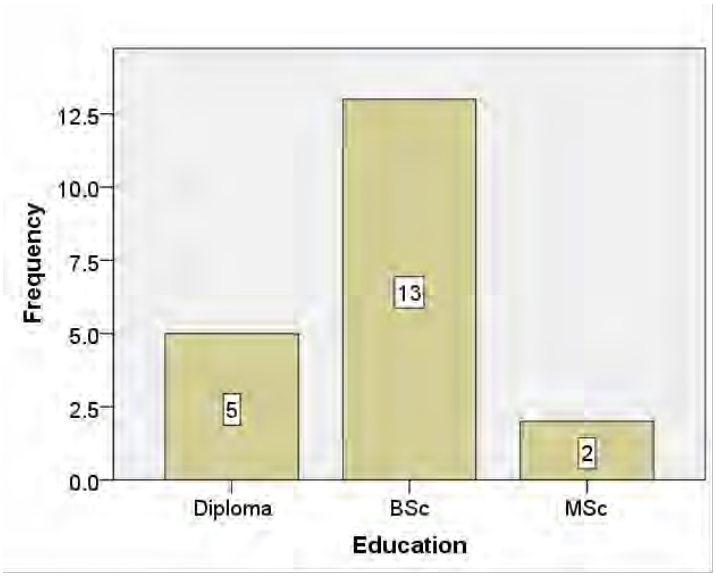

Figure 3. Level of academic training of laboratory professionals in Coagulation/Hematology sections of public hospital laboratories of Addis Ababa, March - June, 2016.

5.2.2 Performance of PT and aPTT

5.2.2.1 SOPs, Specimen acceptability and rejection criteria

According to the results of the data collected form the questionnaires, 12 (60%), 7 (35%) and 1 (5%) of the 20 study participants responded saying that they receive PT/aPTT test requests in a daily, weekly and once in a few days frequency, respectively. All (100%) of the laboratory professionals reported that they have a well written and documented SOP in their respective hospital laboratories to test these requests. However, none of the hospital laboratories had a written policy addressing specimen acceptability and rejection for PT/aPTT during the data collection period of this study. The habit of registering results before dispatching is a well-established good laboratory practice (GLP) among the study participants as all of the laboratory professionals happen to do so.

5.2.2.2 PT/aPTT performance and controls

All 20 (100%) of the study participants reported that they run controls even though the frequency of running controls differs from one laboratory to another (see figure 4 below). Quite majority 70% (14/20) of the hospital laboratories run controls every day. The other 15% (3/20) run controls when a new vial of reagent is opened and the remaining 15% (3/20) run controls when there is a request for a test. Regardless of the irregular practice of running controls amongst themselves, all of the study participants agree that controls should be run every day.

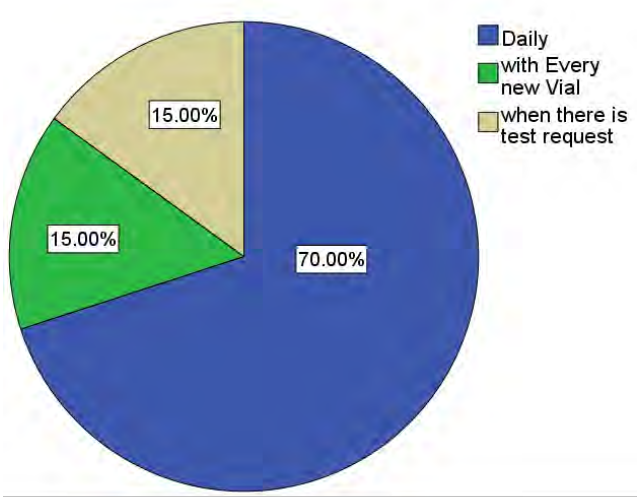

Figure 4. Frequency of running controls in Coagulation/Hematology sections of public hospital laboratories of Addis Ababa, March – June 2016.

5.2.2.3 PT/aPTT performance and Reagents

In order to insure the quality PT/aPTT test result output, it is very important to regularly check the integrity and the quality of reagents. The results of this study indicate that all of the laboratory professionals do regularly check the wellbeing of their reagents. To ensure this, all 20 (100%) of the study participants check the expiry date, 10/20 (50%) carefully noticing any color change and the rest 10 (50%) check their reagents by noticing if there are any agglutinations, precipitations and unusual appearances. While shaking the reconstituted reagents, all the technologists apply a gentle shake.

5.2.2.4 Working space

Eight of the 20 (40%) laboratory technologists' responded saying they are not comfortable with the working space in the laboratory. Among these, 5 (62.5%) complained that the space was too narrow and other 3 (37.5%) reported that the laboratories did not have enough windows for ventilation. The remaining 12/20 (65%) reported that they are comfortable (free) with their laboratory's working space.

5.2.2.5 INR reporting

Even though they do not report INR, all the laboratory professionals indicated that they do realize the significant role that INR reporting plays in standardizing the PT result and enhancing quality health care provision to coagulation patients.

5.2.2.6 Tests to investigate the cause of prolonged PT/aPTT results

None of the four hospital laboratories receive request papers for the investigation of the cause for a prolonged PT/aPTT test result and they perform no tests towards addressing the cause of a prolonged PT/aPTT test results. The majority (18/20, 90%) of the laboratory professionals reported that the reason may be attributed to the clinicians sticking with history taking and clinical diagnosis rather than the laboratory test findings, while 5% (1/20) of them said they do not know the reason and the other 5% (1/20) reacted saying that they think the immature medical provision system of the country is responsible for not receiving any request papers for the investigation of the cause of a prolonged PT/aPTT.

5.2.2.7 Mixing test in public hospitals of Addis Ababa

None of the public hospital laboratories in Addis Ababa perform mixing test. The laboratory professionals mentioned that, not knowing the test at all, having no awareness towards the test and receiving no test requests for mixing test as potential reasons why the test is not being performed in any one of the hospital laboratories (see table 1 below).

Table 1. Reasons for not performing mixing test in coagulation/Hematology sections of public hospital laboratories of Addis Ababa, March – June 2016.

Potential reasons	Frequency	Percent (%)
I Don't know the test	7	35
No awareness	7	35
No request	6	30
	20	100

Academic coverage of mixing test

All of the laboratory professionals reported that Mixing tests were not properly covered during their academic studies. The reason might be attributed to not including the test as part of the curriculum according to the 65% (13/20) and the teachers not covering the test according to the remaining 35% (7/20).

PT/aPTT Training

None of the study participants have ever taken in service refreshment training on the coagulation laboratory testing except during their undergraduate study. Therefore, they all agree that there is a need to conduct such trainings.

5.3 Results of the onsite assessment using checklist

In addition to the questionnaires used to interview the laboratory professionals, a checklist was also used to guide the onsite assessment the performance of PT/aPTT test determination in the hospital laboratories of the four hospitals performing the tests during the study period. The assessment was conducted by the principal investigator of this study.

5.3.1 Storage temperature range

Uniform results have been observed in all the 4 hospital laboratories performing the PT/aPTT tests with regard to storage temperature of reagents. All of them store the reagents in a refrigerator but the temperature of the refrigerator is not monitored at all. The recommended storage temperature for the reagents is 2-8 ^{o}C. To adhere to this temperature range, monitoring the refrigerator's temperature using a thermometer is a key measure which all the 4 assessed hospital laboratories tend to not comply with.

5.3.2 Test tubes and Reagents

All laboratory professionals were found to be mixing the reagents carefully before use. Shaking of the reagents before performing tests was not vigorous or harsh. Another good performance trend among the assessed laboratories was the fact that all were using appropriate sample collection tubes. All the 4 laboratories were using the commercially available 2.7 ml, blue top, vacutainer test tubes containing 3.2% sodium citrate for sample collection. Mixing appropriate sample to anticoagulant ratio is important factor to take into consideration as it influences the final test results. We found out that 3 of the hospital laboratories, take a serious consideration in

making sure the appropriate sample to anticoagulant ratio is measured. They use 1 part of the 3.2% Sodium Citrate (which is already inside the blue top test tube) to 9 part of venous blood sample.

Mixing of the reconstituted tissue thromboplastin prior to pipetting at any step in the test procedure is not an adhered to protocol. Labeling the reagents' opening date is important to mark the end date of the stability time. The technologists in two of the hospitals were observed having properly (eligibly and visibly) labeled the dates. Others claim that the reagents are consumed way before the end of the stability time so they do not label the dates. This is not a GLP complying with the recommended SOPs. The technologists assessed in this study do not use the reconstituted reagent beyond the stability time, 7 days. In fact, the reagents are consumed before the stability time is even approached. This is because there are very few of the public hospital laboratories performing these tests and there is a lot of patient load on the 4 laboratories. Even though it is irregularly handled among the participating hospital laboratories, the technologists gently mix the reconstituted tissue thromboplastin prior to pipetting at any step in the test procedure. The technologists were not observed giving attention to avoid foaming of specimens even though the technologists gently mix the reconstituted reagent with no vigorous shaking.

5.3.3 Centrifugation and covering of tubes

The recommended centrifugation is 1500 RPM for 15 minutes; nonetheless, the hospital laboratories were observed conducting variable centrifugation ranging from 1500-3000 RPM and a varying duration of centrifugation which also ranged from 5-15 minutes. The centrifuges were not continuously monitored and regulated using tachometer. This indicates a practice which does not comply with the SOPs. Covering of the specimens to prevent PH change that may affect the test result was an adhered to protocol while performing PT/aPTT in the hospital laboratories. Since the tubes being used were blue top vacutainer tubes, there was no problem with maintaining this protocol.

5.3.4 Running controls

The technologists who were performing the tests were observed for one full day. During the observation they were running controls prior to performing tests on patient samples. The recommended interval to run controls is, in the morning before starting routine laboratory testing services and also in the afternoon session. The 4 hospital laboratories assessed in this study were observed running controls in the morning before running any patient samples, if there is a request for the test. If not, the technologists run the control when they receive the requests. This is a good practice towards maintaining quality. However this practice is not uniformly maintained. Whenever the control reagent is not available, the technologists simply perform the test without running the controls. For any outlier or unusual test results, the technologists repeat the test to ensure quality test results.

5.3.5 Sample rejection

All of the technologists working in the hospital laboratories which participated in this study were observed rejecting clotted, turbid, hemolysed, icteric or lipemic samples. The technologists perform the tests within two hours on specimens maintained at 22-24 ^{o}C after collection. This leads to a quality test result. The technologists, however, do not preserve the samples for future reference use in case there is a need to rerun or refer back to the samples. Practices related to avoiding foaming of the specimen by the technologist while performing PT/aPPT tests was not a uniformly maintained SOP procedure.

5.3.6 INR reporting

Two hospitals were reporting INR results for patients but none of the remaining hospital laboratories currently report INR at the data collection time of this study.

5.4 Results of the mixing test performed at TASH

A total of 40 plasma samples with a prolonged PT/aPTT results were collected and analyzed for mixing tests at TASH. The general information of the patients were collected and recorded as per the patient checklist (see annex). Proportion of male and female patients in this study was 72.5% (29/40) and 27.5% (11/40), respectively. The median age of the study participants was calculated to be 39.58. The maximum and minimum ages of the study participants were 68 and 19 respectively. The study participants were constituted of 37.5% (15/40) patients being pre-assessed of coagulation state before surgery and all the remaining 62.5% (25/40) were patients

from OPD complaining of bleeding. On an initial determination of the PT and aPTT tests all of these patients had prolonged results with mean PT and aPTT results being 22.98 seconds and 42.13 seconds respectively.

On a second PT and aPTT determination after mixing the patients' plasma with normal pooled human plasma, 45% (18/40) showed correction of results in to the reference interval while the remaining 55% (22/40) did not.

6. Discussion

The study aimed at assessing the practice of PT, aPTT and mixing tests in public hospitals of Addis Ababa, Ethiopia by using a questionnaire, checklist and by performing mixing tests at TASH. The study had established that the performance of PT and aPTT tests does not comply with certain currently recommended guidelines and doesn't adhere to some SOPs as well. Moreover, the provision of PT and aPTT test services in the public hospitals was insufficient as evidenced by the availability of the service only in 4 of the 13 public hospitals during the onsite assessment. Mixing tests are not performed in any of the public hospitals of Addis Ababa.

A survey conducted in the United States of America (USA) agrees that little is known about the various testing practices in a population of hospital laboratories and there is uncertainty surrounding coagulation testing practices. Results of the USA study show an estimated 97.1% of the participating hospitals perform some coagulation laboratory tests. On the contrary only 30.8% (4/13) of the hospitals in the current study reported performing coagulation laboratory tests. This discrepancy clearly shows that coagulation health care provision in our country is in the very early stage whereas it is highly advanced in the developing world in terms of research, coverage of service and quality of the delivered service (28).

Of the 97.1% hospitals in the USA study, 71.6% reported using 3.2% sodium citrate as the specimen anticoagulant to determine prothrombin time (28). In terms of anticoagulant usage, better practice exists in our study as all of the participating hospital laboratories used 3.2% Sodium Citrate as anticoagulant. This is because all of the laboratories use the commercialized blue top citrated vacutainer test tubes. In another survey conducted in Canada, 54% of the participating hospitals use 3.2% sodium citrate while all the remaining used 3.8%. The practice of using 3.8% sodium citrate is in variation to the findings of the current study (47).

Participants in the assessment of PT testing practices in the Pacific Northwest were asked if they collected samples for PT by venipuncture. Of the 260 respondents to this question, 216 (83%) indicated they did while the remaining reported that they collect capillary blood for POC testing. In this study, the majority of respondents (92%) used only the recommended citrate concentration of 3.2%. This finding is similar to our research because all (100%) of the participating hospital laboratories performing PT in the Addis Ababa survey collect venous blood by venipuncture and use a sodium citrate concentration of 3.2%. The commercially

available blue top citrated vacutainer test tubes have contributed for this GLP. In the hospital laboratories of Addis Ababa only venous blood is collected as the POC devices are not available (25).

Of the 216 respondents that collected samples by venipuncture in the Pacific Northwest survey, 202 (94%) said they had a written policy addressing specimen acceptability and rejection for PT testing (25). This finding is in contrast to our current research findings. Though the hospital laboratories of Addis Ababa have written SOPs they do not have a clearly posted specimen rejection and acceptability policy. This might be because the laboratory professionals believe they are familiar with the specimen rejection and acceptability criteria.

An estimated 96.3% and 90% of respondents assayed specimens for aPTT within 4 hours after phlebotomy in both the surveys conducted in USA and Canada. In addition, 89.4% of respondents in the USA survey centrifuged specimens within 1 hour of collection. These testing practices are in accordance with our study because all the hospital laboratories in the current research performed the tests within 2 hours of sample collection. This GLP may be because the samples collected for PT and aPTT are not transported to other laboratories, which is also same in our case (28, 47).

Most (more than 85%) of the respondents in the USA survey rejected coagulation specimens for various appropriate reasons including when medical record numbers were missing from specimen labels and more than 70% of the respondents repeated coagulation tests in circumstances where the results were over prolonged (28). The same practice is also being implemented in the current study as well. The practice of repeating tests with unusual results before dispatching is also observed among the participating hospitals of our current research. This shows that the practice of the laboratory professionals towards enhancing the quality of their test results is well maintained.

The hospital laboratories in the current study were observed using variable centrifugation time and speed. The centrifugation speed used by the laboratories ranged from 1500-3000 RPM. Similar to this, the participating laboratories in the survey conducted in Canada showed an irregular speed and time of centrifugation. The centrifugation speed in the Canadian survey ranged from 1000 – 8,500 RPM. One of the institutions in this survey did not have a maximum

time interval defined and the other one had no policy at all in this regard. Additionally, 93% of the participating institutions in the Canadian survey of aPTT reporting had their centrifuges calibrated using a tachometer or either a stopwatch, among these the 95% of them check their centrifuges on a regular basis (47). On the contrary, the centrifuges in the participating hospitals of the Addis Ababa survey were not observed using a tachometer or any means of calibrating their centrifuges. This might be because of the low concern or level of awareness among the laboratory professionals of the public hospitals in Addis Ababa towards this and may be because the availability of the resource and the materials to keep this quality aspect is limited.

The median number of controls run in one shift (8 hours) in the Canadian study of aPTT reporting was 3. The 4 hospital laboratories assessed in the Addis Ababa study were observed running controls in the morning before running any patient samples, if there is a request for the test. If not, the technologists run the control when they receive the requests for PT and aPTT. This contradicting practice might be because of the easy availability of the control reagents to the developed world rather than the developing world where our country Ethiopia belongs to. (47)

Virtually all of the hospitals in the USA survey used INR to report PT, whereas in our current study only two hospitals report INR (28). This discrepancy may be attributed to the poor performance of the coagulation laboratories in our country and the poor trend of reporting INR. Knowledge gaps due to absence of refreshment trainings as reported by the laboratory professionals during the survey could also be a possible explanation.

According to another Canadian survey of INR reporting in medical laboratories, 55% of client physicians preferred PT results to be reported in seconds rather than INR. In this survey, it was reported that 57% of the responding laboratories utilize some format of INR reporting. In contrast to this, only two of the hospital laboratories in our current research report INR when requested by the client physicians. This shows that further education regarding the INR system for PT reporting is required by both medical laboratories and physicians (48). A follow up update survey to this study in Canada was conducted after 4 years and the results show a remarkable improvement in the percentage of the INR reporting laboratories increased from 57% to 98% even though 35% of the responding laboratories used a thromboplastin with an ISI of 1.2 (49).

According to the survey in USA, 47.5% of the laboratories did not offer mixing studies (28). About 18% of the participating institutions in the Canadian survey performed mixing tests (47) while none of the hospital laboratories in Addis Ababa perform the test. This is clearly because of the low awareness of the laboratory professionals towards the mixing tests here in Addis Ababa due to absence of refreshment trainings as disclosed by the laboratory professionals during the survey. Laboratory professionals' knowledge gaps as well as lack of request from physicians were indicated as possible reasons during the questionnaire based interview.

Though TASH was among the nine hospitals which did not perform PT/aPTT during the study period due to lack of reagents, the service was continued through the current study and those with prolonged values were indicated for the mixing test (the service was sustained thereafter). The mixing study determines if the patient has a factor deficiency or the presence of a factor-inhibiting antibody (50). Accordingly, the current study detected correction of results into the normal range in 18/40 (45%) of the patients and a still prolonged result in the remaining 55% (22/40). The mixing test has been considered as one of the important test in coagulation testing study. Efforts are underway to standardize the procedures and to have consistent set of validated interpretive criteria that represent the most favorable approach to maximizing the utility of the mixing test, and ensure the most accurate diagnosis for patients under investigation (15). International Society on Thrombosis and Haemostasis (ISTH) recommended criteria regarding the cut-off values for the screening, mixing and confirmatory tests for the detection of LA and the mandatory need to perform mixing tests of patient plasma with pooled normal plasma in 2009 (51).

This is an evidence based effort to establish the mixing test, which has been utilized by others in the coagulation testing algorithm (52), at TASH where patients with hematological disorders including bleeding disorders and coagulopathies are referred to.

7. Strength and Limitation

7.1 Strength

- To the best of our knowledge, this research is the first of its kind done in Ethiopia in providing basic awareness on the practice of PT/PTT and mixing tests in the public hospitals of Addis Ababa.
- This study provides evidence for policy makers, hospital management, laboratory management, educators, scientists and medical laboratory professionals in Ethiopia to improve the quality of PT/aPTT performance, conduct further researches and teaching.
- The study clearly demonstrates the advantages obtained from mixing tests if they are introduced in the test menu of our hospital laboratories.
- This study puts us one step further forward in incorporating a simple but neglected laboratory test into the testing system of the laboratories of our public hospitals, thus, improving the health care delivery.

7.2 Limitation

- Private hospitals should have been assessed and included in the study.
- The perspective of clinicians should have been incorporated as well.
- Lupus anticoagulant and individual markers were not measured

8. Conclusion and Recommendation

8.1 Conclusion

- The practice of PT/aPTT tests in the public hospitals of Addis Ababa is insufficient.
- Even though the national guideline states that all hospital laboratories include the PT/aPTT tests in their test menu, only 4/13 of them currently perform the tests. This suggests a very low provision of PT and aPTT test services among the public hospitals of Addis Ababa.
- Out of the four hospitals performing PT, only two of them report INR despite its internationally recommended usefulness in standardizing PT values for OAT monitoring.
- Presence of SOPs and use of correct citrated tube for blood collection are commendable practices to be maintained; however, lack of specimen rejection policy, use of variable centrifugation time and speed, and inconsistent control running time need improvement.
- None of the laboratory professionals had the knowledge of mixing tests. All interviewed professionals (20/20) disclosed lack of refreshment trainings with regards to coagulation tests. Refreshment trainings should include neglected tests like coagulation tests.
- Regardless of their remarkable role in the quality health care provision of coagulation laboratories, mixing tests are not performed in any of the public hospitals of Addis Ababa.

8.2 Recommendation

- Different trainings and workshops should be organized by the concerned bodies to revitalize and refresh the skills, knowledge and experiences of laboratory professionals as well as give emphasis and improve the coagulation laboratory service provision in our country.
- The coverage of mixing tests in the undergraduate curriculum of Medical Laboratory Science should be given attention and that all of our hospital laboratories include PT/INR, aPTT and mixing tests in their test menu.
- Mixing tests should be applied when the clotting time of the basic coagulation assays is outside the reference interval, in order to increase diagnostic efficacy.
- Further research which includes private hospitals and clinicians should be conducted.
- Unlike the rest of the world, coagulation science is an untouched area of research in Ethiopia; thus researchers should get involved with researching the area and contributing to the growth of this science in Ethiopia.
- All these measures will contribute to a more uniform evidence-based coagulation testing practices among the hospitals in view of the recommendations and guidelines for PT, aPTT and Mixing test performance.

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10. Annex

Annex I: Information Sheet for Laboratory Professionals

Principal investigator:	Gelila Biresaw, MLS; AAU.
Advisors/Collaborators:	Aster Tsegaye, PhD; AAU
	Jemal Alemu, MSc, AAU
	Mistre Woldie, MSc, PhD candidate, AAU
	Dr Amha G/Medhin (MD, Hematologist)
	Dr Abdulaziz Abubeker (MD, Hematologist)
	Dr Fissehaye (MD, Hematologist)

Name of institute:	AAU
Funded by:	AAU and Self
Reviewed by:	DRERC (AAU)

Title of the project: *Practice of PT, aPTT and Mixing tests in Public Hospitals of Addis Ababa, Ethiopia*

Why do we need to conduct the study?

PT and aPTT tests are part of the common coagulation tests for diagnosis and monitoring of hemostatic disorders and anticoagulant treatment monitoring. These tests are very crucial as they determine the oral anticoagulant dose and committing error could even lead to death from bleeding or thrombotic complications. The mixing test which is performed by mixing equal amount of standard pooled plasma and patient plasma when PT and aPTT tests are prolonged is indicative of either coagulation factor(s) deficiency if the test is corrected or presence of coagulation factors inhibitor(s) if PT and aPTT tests remain prolonged after the mixing studies. However, no study has been carried out to assess the status of these crucial tests and especially the condition and utility of the mixing test. The main objective of this study is, therefore, to assess the status of these tests among the public hospital laboratories and demonstrate the usefulness of the mixing study by carrying out the mixing tests for patients with prolonged PT/aPTT.

Study procedure

To achieve the planned objective, you will be asked questions about your PT/aPTT practice. In addition a checklist will be used to check how you carry out the tests all the Pre-analytical, analytical and post analytical phases of diagnosis.

For professionals at TASH only: you will be required to communicate the PI any prolonged PT/aPTT tests for the performance of mixing tests. Then you will get results to communicate to the patients for free. The PT/aPTT test will be repeated to validate the result and mixing study will be performed by experienced technologist.

What is expected from me as a study participant?

On volunteer base, the PI will ask you about personal identification information (like age and sex), your level of education, training experience, your overall practice of PT/aPTT and mixing tests. In addition, if you agree to participate in this study, your laboratory's practice will be evaluated using checklist.

How much time will I spent to participate in this study?

You will spend about 10-15 minutes to answer to the questions.

What are the risks of participating in this study?

Since this is a questionnaire and checklist based study, there will be no risk.

How my information is to be kept in secrete?

All information that you give will be used for this study only. All the information will be encoded in the computer and saved with password protection. Hard copies will be safely and carefully locked. All data will be destroyed after the purpose of the study is successfully mate.

What are benefits from participation?

There will be no direct benefit for you from participating in this study. However, as a laboratory professional your satisfaction will be improving the hemostasis laboratory service that you provide to patients. Primarily, your satisfaction will be enhanced by being able to improve the clinical management of patients with bleeding and coagulation disorders and those on anticoagulant therapy.

Incentives and compensation

You will not be paid for your participation.

What are my rights as a participant of the study?

You have the right to withdraw yourself from the study at any time with no consequences at all. You are also welcomed if you have any question for further explanation about the study.

Agreement

After reading about the study procedures and other related issues done in the study, you will be kindly requested to put your signature of agreement. Your signature indicates that your participation is only based on your volunteer participation.

What can I do if I have a question or a problem?

Please direct any question you may encounter during this study to:

Name: Gelila Biresaw

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Email: gelila.afri@yahoo.com

Dr Aster Tsegaye

Mob + 251911696085

Email: tsegayeaster@yahoo.com

Annex II: Consent Form for Laboratory Professionals

Code number _____

Facility Name _____

I have been informed about the study which is aimed at assessing the practice of PT, aPTT and mixing tests in public hospitals of Addis Ababa. For this study true and direct information is needed to fill the questionnaire. A checklist will also be used to check the practice of the PT/aPTT determination in my laboratory. The aims of the study were explained to me.

I am also informed that all the information contained in the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study at any time.

It is therefore with full understanding of the situation that I give the informed consent voluntarily to the research team to properly use the information gathered from me for the study purposes. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to improve the laboratory service we provide for patients who need service for coagulation tests and introduction of an additional test, the mixing test.

Participant's special code _____Signature_____

Name of Interviewer _____ Signature_____Date _____

Please direct any questions or problems you may encounter during this study to:

Name: Gelila Biresaw

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Annex III: Questionnaire

Addis Ababa University

College of Health Sciences

School of Allied Health Sciences

Department of Medical Laboratory Science

The objective of this questionnaire is to assess the Practice of PT/aPTT determination. Your willingness in responding to this questioner will greatly determine the outcome of the study. So, I kindly ask you to respond carefully for each question. I also assure you that any information you give will be handled with confidentiality and will not be used for any other purpose beside this research.

1. Age _____
2. Sex _____
3. Level of academic training
    A. Diploma
    B. BSc
    C. MSc
    D. Other, please specify_____
4. Do you perform coagulation profile tests in your laboratory?
    A. Yes
    B. No
5. Do you perform PT in your laboratory?
    A. Yes
    B. No
6. If you have answered no to question number 5, please specify why

    _____

    _____

7. Do you perform aPTT?
    A. Yes
    B. No

8. If you have answered no to question number 7, please specify why

_____

_____

9. Do you perform mixing test?
   A. Yes
   B. No
10. If you have answered no to question number 9, please specify why

_____

_____

11. Do you have a well written and documented SOP for the PT/aPTT tests?
   A. Yes
   B. No
12. If you have answered no to question number 11, please specify why

_____

_____

13. Do you have a written policy addressing specimen acceptability and rejection for PT/aPTT testing?
   A. Yes
   B. No
14. If you have answered no to question number 13, please specify why?

_____

_____

15. Do you run controls for PT/aPTT?
   A. Yes
   B. No
16. If you have answered no to question number 15, please specify why

_____

_____

17. If you have answered yes to question number 15, how often do you run controls?
    A. Daily
    B. When every new vial of reagent is reconstituted
    C. Whenever there is a test request
    D. Other please specify_____
18. How often do you get requests for PT/aPTT tests?
    A. Daily
    B. Weekly
    C. Once in few days
    D. Not often
    E. Almost never
19. Do you regularly check the wellbeing of your reagent?
    A. Yes
    B. No
20. If you have answered yes to question number 19, please specify how
    A. By checking the expiry date
    B. By carefully noticing any color change
    C. By checking if there are any agglutinations, precipitates and the like
    D. Other ways, please specify_____
21. If you have answered no to question number 19, please specify why
    A. I trust the vendor which provided the reagents
    B. There has never been a problem like this in our laboratory
    C. We strictly adhere to the recommended storage requirements
    D. Other reason, please specify_____
22. While reconstituting the lyophilized reagent, do you shake it?
    A. Gently
    B. Until foam is formed
    C. Other, please specify_____
23. Do you report INR?
    A. Yes B. No

24. Do you know why we need to report INR?
    A. Yes
    B. No
25. If you have answered no to question number 23, please specify why?

    _____

    _____
26. Do you register results before their dispatch?
    A. Yes
    B. No
27. If you answered no to question number 26, please specify why
    A. I do not understand its importance
    B. We get incomplete test request papers which makes it hard
    C. We usually forget it
    D. Other reason, please specify_____
28. What is the number of PT test performed per

    Day_____

    Month _____

    Year _____
29. What is the number of aPTT test performed per

    Day_____

    Month _____

    Year _____
30. Are you confortable /free with the working space in your laboratory
    A. Yes
    B. No
31. If you have answered no to question number 30, please specify why
    A. It is too narrow
    B. There are not enough windows
    C. It makes the laboratory staff prone to infection
    D. Other reason, please specify_____

32. Do you get test request papers for tests performed to investigate the causes of prolonged PT/PTT tests?
    A. Yes
    B. No
33. If you have answered no to question number 32, what do you think is the potential cause
    A. The clinicians treat it with the history they obtain from the patient
    B. Our country's medical provision is not that much mature
    C. I cannot think of any reason
34. What tests do you perform for evaluation of prolonged PT/aPTT tests?

_____

35. Why do you not perform mixing test?
    A. We do not get requests for this test
    B. Other reason, please specify_____
36. Was mixing test properly covered during your academic studies?
    A. Yes
    B. No
37. If you have answered no to question number 36, please specify why
    A. It was not included in the course outline
    B. The teacher did not cover it
    C. Other reason, please specify_____
38. Do you think PT/aPTT controls should be run when a new vial is reconstituted?
    A. Yes
    B. No
39. Have you ever taken in-service refreshment training on coagulation profile tests?
    A. Yes
    B. No

40. Do you think there is a need to prepare in-service refreshment training on coagulation profile tests?

    A. Yes
    B. No

Annex IV: Checklist

1. Does the technologist store the reagents at a temperature of 2-8 degree centigrade?
    A. Yes
    B. No
2. Does the technologist label the reagent opening date?
    A. Yes
    B. No
3. Does the technologist use the reconstituted reagent beyond the stability time?
    A. Yes
    B. No
4. Does the technologist mix the reagent carefully before use?
    A. Yes
    B. No
5. Does the technologist use plastic or siliconised glass test tubes for sample collection?
    A. Yes
    B. No
6. Does the technologist reject turbid, hemolysed, icteric, lipemic or clotted samples that may result erroneous results?
    A. Yes
    B. No
7. Does the technologist use buffered 3.2% (0.109M) sodium citrate as an anticoagulant?
    A. Yes
    B. No
8. Does the technologist mix 1 part of sodium citrate solution to 9 part of venous blood?
    A. Yes
    B. No
9. Does the technologist avoid foaming the specimen?
    A. Yes
    B. B. No
10. Does the technologist centrifuge the sample for 15 minutes at 1500 RPM?
    A. Yes
    B. No

11. Does the technologist cover the specimen to prevent PH changes that may affect test results?
    A. Yes
    B. No
12. Does the technologist run controls?
    A. Yes
    B. No
13. Does the technologist mix the reconstituted tissue thromboplastin prior to pipetting at any step in the procedure?
    A. Yes
    B. No
14. Does the technologist mix the reconstituted reagent until foam is formed?
    A. Yes
    B. No
15. Does the technologist report INR?
    A. Yes
    B. No
16. Does the technologist perform the test within two hours on specimens maintained at room temperature (22-24 ^{0}C) or else preserves the samples?
    A. Yes
    B. No
17. Number of PT tests performed per
    A. Day
    B. Month
    C. Year
18. Number of aPTT tests performed per
    A. Day
    B. Month
    C. Year

Annex V: Information Sheet for Patients

Plasma sample collection for performing Mixing tests on patients' sample which has prolonged PT and aPTT results for the study of the Practice of prothrombin time, activated partial thromboplastin time and mixing test in Public Hospitals of Addis Ababa, Ethiopia February-May 2016.

Greetings!

My name is Gelila Biresaw, a Master of Clinical Laboratory Science student at Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science. This current study is being conducted for the partial fulfillment of Master of Clinical Laboratory Science degree in Hematology and Immunohematology specialty track. The study will find out the Practice of prothrombin time, activated partial thromboplastin time and mixing test in Public Hospitals of Addis Ababa. The study is undertaken by a questioner which has 40 questions and a checklist which contains 18 questions. Both contain clear questions which are tailored to the objective of the research. In addition to the questionnaire and the checklist, mixing test will be performed on 40 plasma samples of patients requested for PT and (or) aPTT and have a prolonged test result during the data collection period of the study. The data from the Questionnaire, the checklist and the mixing test results will be utilized only for the purpose of this research. The filled questionnaires checklists and the mixing test results would not be identified by any unauthorized person and confidentiality will be kept. Once the data is utilized for the research purpose the collected information would be destroyed. You are kindly requested to provide a venous blood so the coagulation laboratory tests can be performed on the sample you provide for the above mentioned rationale. Keep in mind that participation is on a totally voluntarily basis and you will not have any obligation to fill in the questionnaire if you are not willing to do so for any of your reasons.

For any information you can contact

Ms. Gelila Biresaw

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Institutional Review Board (IRB)

Tel: +251-11- 896 1396 and Fax +251-1-1-513099

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Annex VI: Consent Form for Patients

I have read the information sheet above and clearly understood the purpose and anticipated benefit of the research. I hereby assure with my signature below that, I without any coercion or forceful act by the research team, have decided to voluntarily participate in the study to contribute my part by providing venous blood in the effort being made to understand the practice of prothrombin time, activated partial thromboplastin time and mixing test in Public Hospitals of Addis Ababa, Ethiopia.

Unique ID No._____ Signature _____ Date_____

Data collector's name _____Signature_____

Date of data collection _____Start time _____End Time_____

Supervisor's Name_____ Signature_____

I thank you for your cooperation

Please direct any questions or problems you may encounter during this study to:

Gelila Biresaw

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gelila.afri@yahoo.com.

For additional information, please contact Addis Ababa University Medical Faculty Institutional Review Board (IRB) office at:

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P O Box 9086

Addis Ababa, Ethiopia.

E-mail: aaumfirb@yahoo.com

Annex VII: Sample Collection and preparation

Routine venipuncture procedure: Collect the necessary materials which include, needle syringe, test tube, tourniquet, 70 % alcohol wipes, cotton, biohazardous sharp disposal container.

1. Properly identify the patient
2. Position the patient with the arms extended to form a straight line from shoulder to wrist
3. Select the appropriate vain for venipuncture. The larger median cubital, basilica or cephalic veins.
4. Apply tourniquet 3-4 inches above the collection site.
5. Clean the venipuncture site by making a smooth outward circular pass with the 70% alcohol pad.
6. Perform venipuncture
7. Release the tourniquet (before taking off the syringe)
8. Place a gauze pad over the site
9. Place the blood in to plastic or siliconised glass test tubes

Use 3.2% (0.109 M) buffered sodium citrate as anticoagulant and mix by inverting the tube against the stopper. Sodium oxalate, EDTA, and heparin are not suitable. Immediately after obtaining the venous blood sample in a clean venipuncture following the standard operating procedure mentioned above, mix 9 parts of blood with 1 part anticoagulant. Avoid foaming the specimen.

Centrifuge the sample for 15 minutes at 1500 RPM. Remove the plasma using a plastic pipette and store in a plastic tube. Cover the specimens to prevent PH changes that may affect the test results. Turbid, icteric hemolysed or lipemic samples may generate erroneous results therefore must be rejected.

Specimens maintained at 22-24 ^{0}C must be tested within 2 hours. For longer periods of time, plasma should be separated from red cells using a plastic pipette, stored in plastic vial or tube and frozen immediately at -20 ^{0}C for up to 2 weeks or at -70 ^{0}C for up to 6 months. Thaw samples rapidly prior to use at 37 ^{0}C swirl gently and test immediately. Do not refreeze samples.

Annex VIII: Procedure and reagent of HumaClot Duo Coagulation Analyzer

Procedure and reagent preparation for PT determination

Principle:

Single stage PT measures the clotting time of test plasma after the addition of the thromboplastin reagent containing calcium chloride. The reagent supplies a source of tissue thromboplastin which activates factor VII. Deficiencies in factors VIII, IX, XI and XII are not detected by this test.

Humaclot Preparation for PT:

1. Turn on the instrument and wait until the ready LED is lit.
2. Turn on the printer if connected.
3. Select PT as active test.
4. Check calibration.
5. Allow reagent to pre-warm at least for 5 minutes.

Reagent Reconstitution:

Reconstitute the lyophilized reagent with high purity water in the volume stated on the package insert (the manufacturer's instruction). Agitate gently and let stand undisturbed at room temperature for 15 minutes. After reconstitution, the reagent is stable for 7 days at 2-8 ^{0}C and 24 hours at 15-37^{0}C.

Quality Control

Control plasma, such as hemostat control plasma both normal and abnormal, should be tested in conjunction with patient samples. It is recommended that at least one normal and one abnormal control be run at least once each shift and at minimum once per 20 patient samples. A control range should be established by the laboratory to determine allowable variation in the day to day performance of both control plasmas.

Procedure:

1. Pipette 100 micro liter of plasma in to cuvette.
2. Pre-warm plasma for 3 minutes.
3. Transfer cuvette to measuring position.
4. Activate optic (press ―optic" key)
5. Add 100 micro liter of hemostat thromboplastin-SI (measurement starts automatically).
6. The instrument will read for a maximum of 300 seconds. If no clot is detected, the display will read"+++.+ s.".
7. The result is displayed in seconds and INR (53).

Procedure and reagent preparation for aPTT determination

Principle:

The aPTT test is a simple and versatile test which is sensitive to deficiencies of all plasma clotting factors except for factor VII. The aPTT test measures the clotting time of test plasma after the addition of aPTT reagent containing a plasma activator and phospholipid. The phospholipid serves as a substitute for platelets. The mixture is incubated for activation, then recalcified with calcium chloride and clot formation is timed. Deficiencies of approximately 40% and lower of factors VIII, IX, XI, XII and prekallikrein will result in prolonged aPTT. Heparin, in the presence of adequate amounts of AT-III will also result in prolonged aPTT.

Humaclot Preparation for aPTT:

1. Turn on the instrument and wait until the ready LED is lit.
2. Turn on the printer if connected.
3. Select aPTT as active test.
4. Allow $CaCl_2$ to pre-warm at least for 5 minutes.

Reagent Reconstitution: Bring the Hemostat aPTT-EL Calcium Chloride to room temperature prior to use. Mix well by swirling or inversion and keep a stirrer bar in the reagent container during use to avoid the particle activator from settling out. Avoid prolonged heating.

Quality control: Control plasma, such as hemostat control plasma both normal (CPN) and abnormal (CPA), should be tested in conjunction with the patient samples. It is recommended that at least one normal and one abnormal control be run at least once each shift and at minimum once per 20 patient samples. A control range should be established by the laboratory to determine allowable variation in the day to day performance of both control plasmas.

Procedure:

1. Pipette 100 micro liter of plasma in to cuvette.
2. Pre-warm plasma for 1-2 minutes.
3. Add 100 micro liter of reagent to plasma.
4. Incubate for 3-5 minutes.
5. Transfer cuvette to measuring position.
6. Activate optic (press –Optic–key).
7. Add 100 micro liter of pre-warmed Calcium chloride (the measurement starts automatically).
8. The instrument will read for a maximum of 300 seconds. If no clot is detected, the display will read"+++.+ s.".
9. The result is displayed in seconds and ratio (53).

Annex IX: Procedure for Mixing Test

Mix patient sample in 50% proportion or 1:1 ratio to normal pooled plasma, 50 micro liter of patient plasma to 50 micro liter of normal pooled human plasma.

Repeat the PT and aPTT tests as stated on the procedure on the previous annexes.

Procedure for in house normal pooled plasma preparation

1. Collect venous blood from 20 (10 male and 10 female) apparently healthy individuals in separate citrated test tubes using the procedure listed above.

2. Centrifuge each sample at 2360 × g for 15 minutes.

3. Constitute the pool of plasma by mixing 1 ml of the separate plasma in to a separate container of the pooled plasma from the normal individuals.

4. Thaw pooled normal plasma in a water bath at 37 °C for 5 minutes before performing tests.

Annex X: Patient data format

Patient ID	Age	Sex	PT result	aPTT result	After mixing PT result	After mixing aPTT result

Annex XI: Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other university. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate Gelila Biresaw (BSc)

Signature_____

Place: Addis Ababa University, School of Medical Laboratory Sciences, Addis Ababa, Ethiopia.

Date of submission_____/_____/_____

This thesis has been submitted with my approval as the university advisor.

Name of advisor: Dr. Aster Tsegaye (MSc, PhD)

Signature _____

Date of submission_____/_____/_____

This thesis has been submitted with my approval as the university advisor.

Name of advisor: Jemal Alemu (MSc)

Signature _____

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