Turkish Biochemical Society

Guidelines for Preanalytical Phase in Coagulation Tests

Prepared by the Turkish Biochemical Society Preanalytical Phase Working Group
2020-ANKARA
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CONTENT

1. INTRODUCTION

Healthy blood flow within the vascular system is provided by the hemostatic system. Normal hemostasis involves a continuum of coagulation and tissue repair following any damage on the vascular wall. Main elements of the hemostatic system are vascular endothelial cells, platelets, Von Willebrand Factor (VWF), tissue factor, coagulation proteins, fibrinolytic system and anticoagulant proteins. As vascular damage occurs, platelets rapidly form a fibrin plug and prevent bleeding, and the mechanisms which ensures vascular integrity get involved respectively. The balance among the coagulation system, natural anticoagulants and fibrinolytic system is the essential of hemostasis. Disturbance in this balance may cause thrombosis or hemorrhage (1-3).

Hemostasis substantiates in three stages including primary, secondary and tertiary stages. Primary hemostasis contains adhesion and aggregation taking place over vascular endothelia, platelets and VWF and secondary hemostasis contains fibrin formation by plasma coagulation factors. Tertiary hemostasis is the process of consolidation through fibrin polymer formation and fibrinolysis (1, 2).

Hemostatic disorders are evaluated using a series of diagnostic screening tests in medical laboratories. These tests are analyzed with several techniques and methods in modern hemostasis laboratories.

Basic test for primary hemostasis is platelet count. Prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time (aPTT) which are also known as tests for secondary hemostasis are the tests frequently studied in clinical laboratories. Thrombin time (TT) is among the tests studied even if it is not studied as frequent as the others (2, 3). PT/INR, aPTT and TT are the tests which are sensitive to deficiency or absence of one or more procoagulant factors. While PT/INR is sensitive to Factor (F) I, FII, FV, FVII and FX, aPTT is sensitive to FI, FII, FV, FVIII, FIX, FX, FXI and FXII. Deficiency or absence of one or more factors can cause bleeding tendency, hemophilia A or B. Besides, PT/INR test is important in monitoring oral anticoagulant therapy such as warfarin which is a vitamin K antagonist. aPTT can be used to evaluate intrinsic and common path functions; this test is influenced by high molecular weight kininogen, prekallikrein, FVIII, FIX, FXI and FXII levels. Nevertheless, excess of some procoagulants (FVIII, FIX and FXI) can lead to thrombophilia. In addition to these test, in coagulation laboratories, hemostasis systems of individuals are evaluated with factor levels, fibrinogen, D-dimer, FII, FV, FVII, FVIII, FIX, FX, FXI and FXII, factor inhibitors, protein S and protein C, anti-FXa, activated protein C resistance (APCR), lupus anticoagulant (LA) screening and verification, platelet function tests and genetic tests (2, 3).

1.1. Why is Preanalytical Phase Important for Coagulation Tests?

Using modern coagulation devices and taking necessary precautions for quality assurance in coagulation tests, enhance the reliability of the tests and provides to decrease analytical errors. Analysis process is carried out in good quality by using internal quality control materials and external quality assessment protocols. But still inappropriate test results are reported, and this is caused by the processes other than analytical phase. As in all the other laboratory tests, also in coagulation tests, preanalytical phase is the stage where laboratory errors are encountered most frequently. Most of these errors are controllable and correctable. Failure in sampling from the right patient at the right time and in collecting the right specimen is the leading source of preanalytical phase errors. Preanalytical errors that may occur will influence the analytical phase and cause erroneous reporting for the patient's test results. Errors in coagulation tests have vital importance for, especially in patients receiving anticoagulation therapy, medication dose is adjusted according to these test results (3).

This national guideline includes preanalytical procedures related to test order, blood sampling, sample transportation, processing, and storage of plasma samples for screening and special coagulation tests in which plasma is used. This guideline describes each process step in detail and includes recommendations for coagulation tests. Also, this guideline evaluates physiological states and drug interactions that can have effects on coagulation tests. Molecular diagnostic tests and platelet function tests used in evaluating the hemostasis system are outside the scope of this guideline.

2. DEFINITIONS AND ABBREVIATIONS

Antithrombin III (AT-III): It is a protein that inhibits coagulation by activating thrombin. AT-III which is synthesized in liver is the plasma inactivator providing inhibition of thrombin and coagulation factors, FIX, FX, FXI, FXII, and their active forms and serine proteases of coagulation such as prekallikrein.

Anti-factor X (Heparin): Heparin is a polysaccharide which is composed of iduronic acid and D-glucosamine containing sulphate group in various positions. Both standard and low molecular weight heparin inactivates FXa by binding to antithrombin.

Activated protein C resistance (APCR): It is used as a screening test for APCR developing primarily as a result of Factor V Leiden mutation, and infrequently as a result of some rare mutations emerging in the structure of Factor V. It is characterized by a low anticoagulant response of the patient's plasma following the addition of APC.

Activated Partial Thromboplastin Time (aPTT): It is used to determine functions of the factors in intrinsic and common pathways. It is found to be prolonged in deficiencies of factors in these pathways or in the presence of antibodies developed against them. In this test, the time until the formation of fibrin clot in intrinsic pathway is measured by adding calcium and partial thromboplastin (tissue factor) to plasma.

D-dimer: Plasma D-dimers are the fibrin products rising from the effect of plasmin on the cross-linked fibrin D fragments. D-dimer (DD) arises as a result of degradation of a fibrin clot by plasmin; fibrin clot is formed by cross links related to the activation of coagulation system by any cause.

Factor I (fibrinogen): Fibrinogen which is the soluble primer of fibrin is a 340 kDa protein synthesized in liver. Fibrinogen is the primary substrate in fibrin clot formation. It is a glycoprotein which is formed in liver. With the effect of thrombin, it changes to a visible clot that is fibrin.

Factor II (prothrombin): It is synthesized in liver. Only after carboxylation with vitamin K, it activated and converted to thrombin. It takes role in the reaction converting fibrinogen to fibrin in the last stage of the coagulation cascade.

Factor III (tissue factor, thromboplastin): It is the cofactor of FVIIa. Factor III, also called platelet tissue factor, FIII, or CD142, is a protein encoded by the FIII gene, present in subendothelial tissue and leukocytes. Its role in the clotting process is the initiation of thrombin formation from the zymogen prothrombin.

Factor IV (calcium): It provides coagulation factors to bind phospholipids.

Factor V (labile factor): It is synthesized in liver; %20 of it is released from platelets. It plays the cofactor role in the conversion of FII to FIIa. It has no effect on Vitamin K activation. It undergoes proteolysis with protein C/S complex.

Factor VII (stabile factor): It is synthesized in liver. It is activated by making complex with tissue factor. For its activation, carboxylation with vitamin K is needed.

Factor VIII (antihemophilic factor [AHF], antihemophilic globulin, antihemophilic factor A): It is synthesized in liver and endothelial cells of other organs. It is not affected by liver failure and vitamin K deficiency. It is the essential factor of the intrinsic coagulation pathway.

Factor IX (plasma thromboplastin component [PTC], Christmas Factor, Antihemophilic Factor B): It is synthesized in liver. It needs vitamin K to be activated. It is the essential factor of the intrinsic coagulation pathway.

Factor X (Stuart-Factor, Prower Factor, Stuart-Prower Factor): It is synthesized in liver. It needs vitamin K to be activated. It is the essential factor of the intrinsic coagulation pathway. It activates FII and makes prothrombinase complex with Factor V.

Factor XI (plasma thromboplastin antecedent [PTA], antihemophilic Factor C): It is synthesized in liver. It activates FXII and FIX in the intrinsic coagulation pathway.

Factor XII (Hagemen Factor, surface Factor, contact Factor): It is synthesized in liver. It activates FXI, FVII and prekallikrein. .

Factor XIII (Fibrin stabilizing factor): It converts fibrin monomers to fibrin polymers.

International Normalized Ratio (INR): For plasma or complete blood sample collected from a patient who is under anticoagulation therapy for a long time, it is a ratio that provides to standardize prothrombin time using prothrombin time reactive having a known International Sensitivity Index (ISI) value.

ISI: ISI value is obtained from calibration of the thromboplastin which is used in prothrombin time test according to the primary international reference material (combined human thromboplastin coded 67/70).

Lupus anticoagulant (LA) screening test: It is the test used in screening autoantibody (LA) existence that prolongs phospholipid-dependent coagulation tests.

LA confirmatory test: In case of lupus screening test prolongation, it is used for confirmation of the existence of lupus anticoagulant.

von Willebrand faktör (VWF): vWF is synthesized in endothelial cells and megakaryocytes as unbranched polypeptides. vWF prevents platelets to adhere to vascular wall (adhesion) by binding to collagen and GPIb receptor in the subendothelial tissue of the damaged vessel.

Prothrombin Time (PT): It is the test used for evaluating common and extrinsic pathways of coagulation. In this test, time to fibrin clot formation in extrinsic pathway is measured by adding calcium and thromboplastin (tissue factor) to patient plasma.

Protein C (Autoprothrombin IIA and coagulation factor XIX): It is a vitamin K-dependent proenzyme synthesized in liver, found in plasma, and having anticoagulant effect.

Protein S: It is a vitamin K-related plasma protein essentially synthesized in liver. In the coagulation process, it displays a basic anticoagulant function by playing a cofactor role for APC.

Thrombin Time (TT): It indicates the conversion time of fibrinogen to fibrin in the last stage of coagulation system.

3. RATIONAL COAGULATION TEST ORDER

Inappropriate test order is a source of preanalytical errors. In recent years, coagulation laboratories are confronted as a research area which develops rapidly and probably causes "excess" or "inappropriate" test orders (5). It is essential to order a test at the right time, but timing is usually overlooked (6, 7). For instance, following a thrombotic event, natural anticoagulant loss (depletion) might appear; for this reason tests performed just after thrombosis can lead to erroneous assessments. FVIII can rise after thrombosis and if only aPTT based lupus anticoagulant screening test is used, this can lead to skipping LA identification. Unlike this, heparin treatment can affect ATIII measurement and warfarin can affect protein C and protein S levels, concomitant use of heparin and warfarin can affect natural anticoagulant measurements as in APCR test. Heparin and warfarin treatment can have an effect on appropriate LA detection. Studies suggest that 1/3 of the tests requested for evaluating thrombophilia is ordered for patients receiving warfarin and/or heparin or that the sample is contaminated with heparin and therefore these samples have a high potential of erroneous diagnosis. When viewed from another aspect, over 80% of abnormal thrombophilia test results can be the reflection of inappropriate test order during anticoagulant treatment (7). Thus, keeping the laboratory in ignorance about the diagnosis and anticoagulant treatment of the patient concurrently with the test order can cause to misdiagnosis and unnecessary test repeats (8).

Recommendation:

- Each laboratory should develop an information form for specific coagulation tests.
- This form should be submitted to the laboratory together with test order or electronically.
- In this form, along with the identity and demographic information about the patient, clinical and laboratory information, provisional diagnosis, diagnosis, medications that the patient receiving should be specifiable (4, 8).

4. VENOUS BLOOD SAMPLING FOR COAGULATION TESTS

4.1. Time of Sampling

Time of sampling in coagulation tests is important. Preferred and recommended time for sampling is after 8-12 hours of fasting early in the morning. It is recommended to make the patient rest in sitting position for at least 10-15 minutes before venous blood sampling. Sampling should be performed between 07:00 and 09:00 am and, if the patient is smoking, at least 30 minutes following the last smoking (9-12). If the tests are requested for the patient to monitor coagulation and fibrinolysis it is important to draw blood samples at the same time of the day, if possible (13).

Recommendation:

Venous blood sampling for coagulation tests should be performed;

- Following 8-12 hours fasting between 07:00 – 09:00 am,
- If the patient is smoking, at least 30 minutes following the last smoking,
- For treatment monitoring, always at the same time frame.

4.2. Blood Sample Tubes

Tubes used for coagulation tests are identified with a light blue cap and contain citrate as anticoagulant (13). Samples for coagulation-based tests should be drawn into tubes that have surfaces not containing activator (4). It is recommended to draw venous blood samples directly into the glass or plastic tubes containing an appropriate anticoagulant using a blood collection system (4). In the market, there are a lot of tube manufacturers. Despite similarities among the manufacturers related to the tube citrate concentrations, it should be kept in mind that there are structural differences among the tubes (glass, plastic, bilayer, single layer, etc.) and there can be potential differences among the results of coagulation analyses performed in plasmas obtained from bloods drawn into different tubes (14).

4.2.1 Glass Tubes

Surfaces of the tubes manufactured from high quality glass have the feature of activating coagulation cascade. In order to prevent this, glass tubes are siliconized (4,15). While glass tubes are more frequently used for coagulation tests in the past, plastic tubes supersede those glass tubes because glass tubes are broken easily, have low resistance to pressure, so they cannot stand to centrifugation and put laboratory workers' safety at risk (16).

4.2.2. Plastic Tubes

Plastic tubes are flexible, resistant to high-speed centrifugation, safer for workers along with their feature of burnability thus ensures decreasing burden of medical waste, so they are less harmful for the environment (17, 18). Due to these reasons, plastic tubes are more frequently preferred and more commonly used today.

Plastic tubes are produced from polyesters like polyethylene terephthalate (PET), polyolefins like polyethylene and polypropylene (PP), polyacrylic, polytetrafluoroethylene, polysiloxane, polyvinyl chloride, polyacrylonitrile and polystyrene (19). Nevertheless, plastic tubes have more gas permeability than glass tubes. PET that has the features of infrangibility and long-term vacuum is prevalently used in manufacturing blood collection tubes. And PP is another plastic material which is preferred for providing to keep fluid anticoagulant volume and concentration due to having less water permeability. For this reason, certain plastic tubes have a double-layer structure preventing evaporation; while internal PP layer minimizes the evaporation of citrate solution, external PET ensures better observation of tube filling. PP and PET prolong the shelf-life of combined double-layer tubes and at the same time provide to keep anticoagulant volume (20).

Recommendation:

- For coagulation tests, plastic blood collection tubes can be used.
- Double layer plastic blood collection tubes are recommended.

4.2.3 Tube Volume and Number to be Used

Various national and international tube manufacturers offer products in different volumes (e.g. 2.7 mL, 2.8 mL, 1.8 mL, 4.5 mL etc.) either glass or plastic in order to use in coagulation tests. Each laboratory should specify number and volume of the tubes to be used in the patient in accordance with the coagulation-based test panel. This issue is very important particularly in hospitalized and newborn patients regarding probable anemia. The best application for tube size is to use the smallest tube. But it should provide sufficient amount of plasma for all ordered tests and approximately 50% of the obtained plasma should be kept for additional tests if required (14).

Recommendation:

Each laboratory should specify number of tubes and the type of the tubes to be used in accordance with the test panel according to its own conditions.

4.3. Tube Additives: Anticoagulants

In coagulation tests, the most frequently used anticoagulant is citrate. Citrate contained in the tube prevents clot formation by binding calcium existing in the plasma rapidly in order to form a soluble complex. In coagulation-based tests, calcium is added into the reaction media subsequently and the duration until clot formation is monitored and reported. For this reason, citrate concentration in the tubes used for coagulation-based tests constitutes one of the key factors that can influence the results of coagulation-based tests (21,22).

The anticoagulant recommended for coagulation tests is 105-109 mmol/L (3.1%-3.2%, frequently expressed as 3.2%), dihydrate form of trisodium citrate ($Na_3C_6H_5O_7.2H_2O$) which is buffered or not buffered. However, also 129 mmol/L (3.8%) trihydrate form of trisodium citrate can be used (4). Laboratories should standardize sodium citrate concentrations (3.2% or 3.8%), because different citrate concentrations vary in terms of normal values. Particularly, aPTT and PT results which are out of the reference range vary in different citrate concentrations. Clotting time tends to be longer in 3.8% sodium citrate concentration than 3.2% sodium citrate concentration, because it binds more assay added calcium during the test (23, 24). For instance, if reference ranges for PT, aPTT and fibrinogen are determined according to the tubes containing 3.2% sodium citrate, in samples collected in 3.8% tubes, PT and aPTT results can be high and fibrinogen results can be low (23, 25). Tubes with 109 mmol/L bufferred sodium citrate containing theophyline, adenosine and dipyridamole (CTAD) which are used to decrease platelet activation can be used in coagulation tests. Other anticoagulant agents (oxalate, heparin or EDTA) cannot be used in any way (4).

When laboratories need to change the coagulation tests tubes, it is indicated to carry out a parallel study with the purpose of evaluating the effects of different tubes or manufacturers on plasma-based coagulation tests. Effects of differences or variations that are due to tube containers or manufacturers may not be seen if the coagulation test results are in the reference range. Nevertheless, test results can display significant differences in samples which have prolonged values (4,26). For this reason, comparative studies should be conducted not only within the normal range samples but also with samples which resulted in pathological results.

Recommendation:

- It is recommended to use tubes with 105-109 mmol/L (3.2%) citrate concentration for coagulation tests.
- Laboratory specialists should keep in mind that there can be reference range differences due to citrate concentrations in the same method, so they should correct reference ranges.
- In case of tube change, laboratory comparison studies should be conducted, and these studies should be carried out with samples with normal and pathological values.

4.3.1 Blood/Anticoagulant Ratio

For coagulation tests, blood/anticoagulant citrate ratio is 9:1. Insufficient or excess filling of blood collection tubes changes this ratio, leading to erroneous test results (27-29). When blood volume in the tube decreases, blood/anticoagulant ratio falls under 9:1 ratio and coagulation time prolongs. This effect is similar in 3.2% and in 3.8% sodium citrated tubes (23). In order to ensure required blood/anticoagulant ratio for correct test results, one should pay attention to draw the blood amount specified by the manufacturer (on the fill line). Overall approach is to fill the tube up to +10% of the nominal fill line (90%-100%) with blood (4). Pediatric (2 mL or less volume) blood collection tubes are more sensitive to fill volume variability than 5 mL tubes, such that it is reported erroneous and clinically significant high INR results in cases of fill volumes less than 90% (30). Open collection should be avoided in pediatric tubes either, as citrate is volatile and can jeopardize maintaining the correct blood additive ratio by spilling during open collection. Capillary collection should be avoided because of the effect of tissue thromboplastin, and only venous blood collection should be done. In tubes of different manufacturers, appropriate fill volume falls on the line where only "half of the tube is full" (Figure 1). Phlebotomists are usually instructed to fill the tube "completely" and when they draw blood more than the acceptable volume, this can be confronted as a problem. What matters is the fill volume mark on the tube. Excessively or insufficiently filled up the tubes must not be accepted for the test and must be refused (Figure 2).

It needs to be kept in mind that; for the easy detection of incomplete filling some tubes may have minimum fill level indicator (90%) instead of nominal fill level indicator (100%). In this case all the samples with a volume under the minimum fill level indicator (<90%) must be rejected.

Recommendation:

- For providing correct blood/anticoagulant ratio, it should be paid attention to draw the amount of blood up to the fill line which is specified by the manufacturer of the tube.
- It should be paid attention that tube fill line is determined in such a manner that it ensures the correct blood to citrate ratio (9:1), not according to the volume of the citrated tube (Figure 1).
- Open collection should be avoided in pediatric tubes either, as citrate is volatile and can jeopardize maintaining the correct blood additive ratio by spilling during open collection.
- Capillary collection should be avoided because of the effect of tissue thromboplastin, and only venous blood collection should be done.

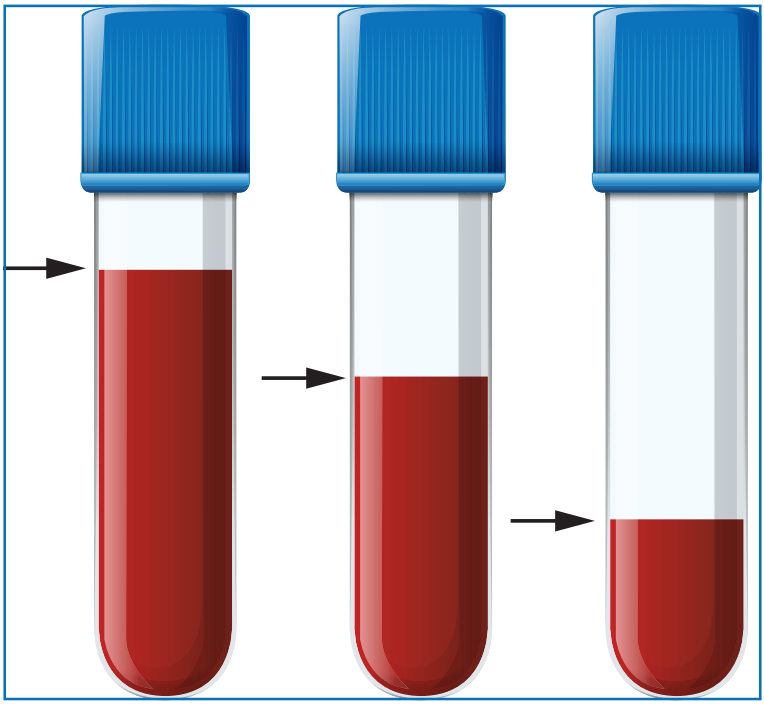

Figure 1: Fill line is determined in such a manner that it ensures the correct blood citrate ratio, not according to the volume of the citrated tube

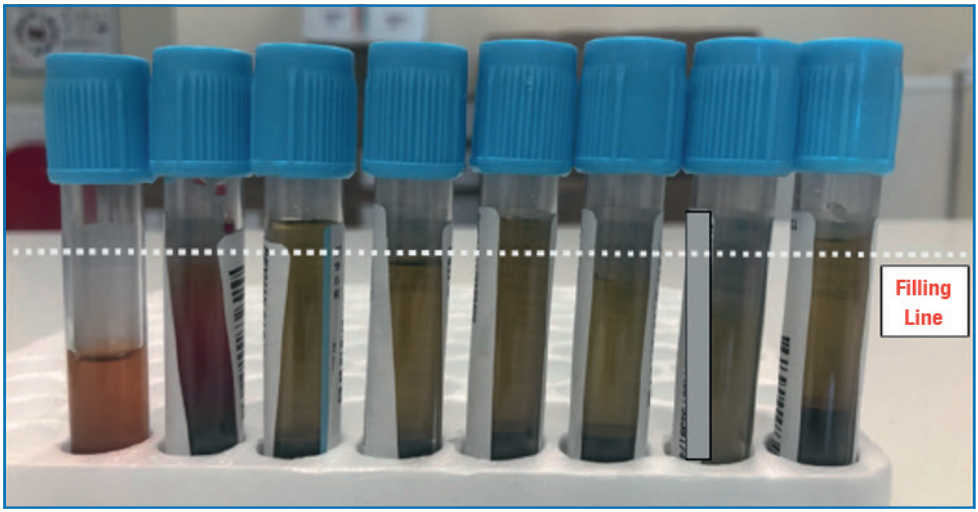

Figure 2: Correct and erroneous blood sampling according to the fill line

4.4. Blood Collection Needle

Manufacturers of blood collection set produce needles together with blood tubes. New production techniques are developed by the manufacturers for to get rid of roughness on the internal surface of the needle that can cause hemolysis and in vitro coagulation activation in the needle. During venous blood sampling, size of the blood collection needle should be specified according to the blood volume to be drawn, age of the patient and vein diameter of the patient. 19-21 G (gauge) size needles are ideal for collecting blood from antecubital vein; smaller size needles (21 G and over) can be used in newborns and infants and adults whose veins are thin (4). It should be kept in mind that large G size number is for needles with small diameters and small G size number is for large diameter needles. >25G size numbered needles should not be selected because these needles can cause hemolysis or platelet activation leading to erroneous results in coagulation tests (31). And, for smaller gauge, larger diameter needles, hemolysis can develop due to turbulence in whole blood samples (4).

Recommendation:

- In venous sampling, phlebotomist should determine blood collection needle size number according to the required blood volume, age of the patient and patient's vein diameter.
- For coagulation tests, 19-21G size numbered blood collection needles should be used. Smaller size numbered (>21G) needles can be used in children and individuals who have thin veins.

4.5. Venous Sampling for Coagulation Tests

For coagulation tests, it is highly important to collect the sample from peripheral veins with minimal vascular damage and, if the patient has intravenous catheter, from the utmost site from the catheter. Stages to be followed for verification of the patient's identity, tourniquet time, time of filling the tube for coagulation tests and mixing the tube are stated in the "Turkish Biochemical Society (TBS) Guidelines for Venous Sampling" in detail (32).

4.5.1. Special Cases of Venous Sampling

In patients whose veins are not convenient for blood collection, blood specimen can be obtained through intravenous catheter (central or peripheral). But the laboratory must be informed definitely about this condition with information added to the test order form. While the results are interpreted, it should be kept in mind that heparin contamination or sample dilution can occur.

It should be avoided to collect blood sample from heparinized catheter. However, if there is no other alternative, prior to blood collection for coagulation tests it is recommended to dispose the first 5 mL blood or catheter dead volume which is equal to the six fold of the catheter line volume after flushing the catheter with normal saline (4, 33).

4.5.2. Blood Sampling Staff

It is recommended to perform venous blood sampling for specific coagulation tests by trained nurses and phlebotomists. Blood sampling staff is needed to be trained particularly about order of draw for citrated tubes, features of the equipment to be used and fill volume of the tube.

Recommendation:

- Blood sampling staff should be trained about specific coagulation tests at certain intervals (once in 6 months).

4.5.3. Order of Citrated Tube Draw

In patients for whom coagulation tests are ordered along with the other laboratory tests, the order of draw for sampling in tubes with different features is critically important. After entering the vessel, in order to minimize the probability of contamination from the other tubes containing additives and micro-clot formation by clot activation that have the potential of effecting coagulation tests, blood sample collection in citrated tubes should be on the first rank as stated in the Guidelines for Venous Sampling. If blood culture is also ordered for the patient, citrated sample should be collected in the second rank following the blood culture sample (32).

In the studies conducted, it is demonstrated that discard tube practice (certain amount of blood is collected in another tube and discarded) is not needed for routine coagulation tests and D-dimer (4,13). Discard tube in coagulation tests should only be used in blood collection procedures performed by using butterfly needle set in order to eliminate the volume difference constituted by the air in the set tubing (4,34).

Recommendation:

- In patients for whom coagulation test is ordered along with other laboratory tests, blood should be collected in the citrated tube in the first rank.

4.5.4. Mixing The Citrated Tube

Whatever the type of the tube used for blood sampling, all the tubes containing anticoagulant should be mixed by turning the tube upside down 3-6 times just after drawing the blood in order to make the sample completely mix with the anticoagulant, unless any different procedure is recommended by the manufacturer. Tubes should not be agitated or shaken vigorously. Excessive stirring can cause hemolysis and/or platelet agglutination and activation, and finally can lead to erroneous results in coagulation tests (4).

Recommendation:

- Unless any different procedure is recommended by the manufacturer, tubes containing anticoagulant should be mixed 3-6 times after drawing the blood.

4.5.5. Correcting Citrate Concentration According to Hematocrit Value

In samples with high hematocrit values, the required 9:1 blood/anticoagulant (or plasma/anticoagulant) ratio decreases. In this case, citrate is relatively high in obtained plasma. High citrate concentration causes excess calcium binding and dilution effect due to existing liquid anticoagulant in coagulation based measurements; this in turn causes prolongation of clotting time (4).

In patients with ≥55% hematocrit value, correction should be done by removing a certain volume of liquid citrate in the sample tube. The amount of required citrate in sample tube is calculated with the following formula (4,35).

Citrate volume needed to remain in the tube = $(1.85 \times 10^{-3})(100\text{-Hct})(V\text{Blood})$,

- Hct= Hematocrit value of the patient
- V= Blood volume to be drawn (if 3 mL tube is being used, the volume is 2.7 mL)
- 1.85×10^{-3},refers to a constant specified considering citrate volume, blood volume and citrate concentration.

After blood is collected with appropriate citrate volume, sample is mixed and plasma is separated. It is reported that when hematocrit value is <25% coagulation tests are not affected, and accordingly it is not needed to correct citrate concentration (36).

- For example, in a 3 mL coagulation sample tube containing 0.3 mL of sodium citrate and 2.7 mL of blood the corrected volume of sodium citrate required for the hematocrit values of 55%, 60%, 65%, 70%, is given in Table 1.

Hct%	Citrate volume to remain in the tube (mL)	Required citrate volume (mL)	Citrate volume to be removed (mL)
55%	$=(1.85X10^{-3})$ X (100-55) X 2.7	0.225	0.075
60%	$=(1.85X10^{-3})$ X (100-60) X 2.7	0.200	0.100
65%	$=(1.85X10^{-3})$ X (100-65) X 2.7	0.175	0.125
70%	$=(1.85X10^{-3})$ X (100-70) X 2.7	0.150	0.150

Table 1. Citrate concentrations corrected according to hematocrit values.

Recommendation:

- For patients whose hematocrit values are ≥55%, coagulation test results should not be given and a comment should be given with the report: "Due to high hematocrit value (XX%), anticoagulant (citrate)/blood ratio which is needed for coagulation tests cannot be reached. Inappropriate anticoagulant/blood ratio causes variations in the test results. It is suitable to perform the test again with a new sample. Please communicate with the laboratory specialist".
- After the coagulation tests performed, a comment should take place in the report: "Coagulation tests were performed in plasma samples having corrected citrate concentration due to the patient's high hematocrit value (XX%)"

5. TRANSPORTATION OF SAMPLES

5.1. Sample Transportation for Coagulation Tests

Samples should be delivered to the laboratory as soon as possible after venous sampling for coagulation tests at room temperature (<4 hours) Before transportation, samples should be checked for identity verification, patient and worker safety conditions and their stability during transport. (4). With respect to time, studies have shown that this rule can be applied to the coagulation tests except PT and D-dimer. For PT and D-dimer, sample is stable at room temperature for 24 hours before or after centrifugation (4,37). For all coagulation tests, after sample collection, tubes should not be uncapped. This ensures transportation safety of samples and decreases CO2 loss that causes pH increases and prolongation of PT and aPTT. Samples should be transported by a transport bag which keeps blood tubes fixed and upright position and attention should be paid not to shake the samples during transportation. (Figure 3) (38,39).

Figure 3: Appropriate and inappropriate transportation of the blood tubes

While samples are transported with a pneumatic system, in order to prevent platelet activation and protein denaturation, sample tubes should be protected from oscillation and rapid, tight turns (4).

5.2. Transportation Temperature

During the transportation of the samples, excessive temperatures (high and low) should be avoided. Whole blood samples should be transported at room temperature (18-25 ° C) for all coagulation tests. It is not recommended to transfer the samples at cold medium (2-8 ° C), due to activation of Factor VII and loss of vWF and platelet integrity. (4, 37, 40, 41).

5.3. Transportation Time

Coagulation proteins (especially FV and FVIII) can be degraded in vitro within the period from sampling to analysis (39). The rise in temperature and delays in the time from sampling to analysis accelerates the loss of these factors and causes pseudo-prolongation of PT and aPTT in most patients. In patients who are not receiving heparin, for aPTT and other coagulation tests, non-centrifuged and centrifuged samples should be tested within the first 4 hours immediately after sampling. If the distance of transportation is long after centrifugation, plasma should be separated and then should be delivered to the laboratory within the first 4 hours (42).

6. SAMPLE REJECTION CRITERIA

Samples that must be rejected necessarily:

- Samples collected in improper tubes (samples containing inappropriate anticoagulant or not containing anticoagulant)
- Samples collected into expired tubes
- Samples with insufficient/excessive volume where required blood/anticoagulant ratio is inappropriate.
- Samples with clots (39).

Recommendation:

- Recommendations above include imperative rejection criteria for coagulation tests. It is recommended that each laboratory should develop its own rejection criteria on its own terms.

Samples obtained in which type of tube is not known and separated from its cells (serum, plasma, other) should be rejected, since these samples resemble each other. Rejected samples may cause delays in diagnosis and treatment follow up of the patient, therefore blood collection staff must be informed and trained about the sample rejection criteria.

7. SAMPLE PROCESSING

Samples for the coagulation testing should be checked with respect to correctness of the barcode, expiry date of the tube, sample volume/amount and existing clot before centrifugation. Negativity in one of these check points requires rejection of the sample (13,29). It may be difficult to control each sample with respect to clot existence. Even sometimes, the existence of micro-clots that affect the coagulation test results may overlook. Therefore, it should be kept in mind that unexpected PT and/or aPTT results can be increased due to the existence of clot in the tube (37).

Recommendation:

- Staff working in the sample accessioning unit should check delivered sample with respect to correctness of the barcode, expiry date of the tube, sample volume/amount and clots before centrifugation.
- Staff working in the sample accessioning should be trained on regular intervals (once in 6 months) on these issues.

7.1. Centrifugation of Coagulation Samples

Coagulation measurements such as PT, aPTT, TT, fibrinogen and D-dimer are performed in platelet-poor plasma (PPP) which is obtained by centrifugation of citrated whole blood sample at room temperature (18-25oC), at 1500 g for 15 minutes. Overall, platelet count in the sample obtained should be <10 x 109/L (10 000/µL) (4). Efficiency of centrifugation for preparing PPP, as a part of quality assurance process, can be established by platelet counts in the plasma sample by counting it in routine blood count devices once a year. In order to prevent plasma sample to be contaminated with platelets and other cells temperature controlled, swing-bucket and brakeless centrifuges should be used. Efficiency for obtaining PPP should be verified and documented by measuring platelet count in the first set-up and once in every six months or after maintenances of centrifuge (4). Centrifuge maintenance should be in regular intervals and it should be checked if there is vibration (while accelerating and/or decelerating) due to lack of maintenance or not (43).

It is suggested that, particularly for emergency routine coagulation tests, plasma obtained by higher centrifugal force (>1500 g) and shorter time (<10

minutes) should be used (44). But it should be kept in mind that high centrifugation speeds can cause hemolysis in red blood cells and activation in platelets; this in return can exert an influence on coagulation tests (4, 39). It is also reported that aPTT, PT/INR, fibrinogen and D-dimer tests are not affected by a platelet count up to >200x109/L (200 000/µL) (45, 46). However, these samples should not be stored for future analyses by freezing (47).

Particularly for specific coagulation tests that will be analyzed after freezing (such as protein S and C, APCR, LA screening and validation, antiphospholipid antibody test, VWF, coagulation factors) it is important that the platelet count is <10x109/L (10 000/µL) (42).

Recommendation:

- While preparing the plasma sample, temperature controlled, swing-bucket and brakeless centrifuge should be used.
- Efficiency for obtaining PPP of the centrifugation should be verified and documented in the first set-up and once in every six months or after maintenances by measuring platelet count.
- Centrifuge maintenance should be in regular intervals and it should be checked if there is vibration (while accelerating and/or decelerating) due to lack of maintenance or not
- Citrated whole blood sample collected for coagulation tests is recommended to be centrifuged at room temperature (18-25oC) at 1500 g for 15 minutes.
- For emergency PT, aPTT and fibrinogen tests, higher centrifugal force (>1500 g) and shorter time (<10 minutes) should be used.
- To prepare PPP with a residual platelet count ≤10x109/L, double centrifugation procedure is recommended.

7.2. Allocation of the Sample

While the sample to be aliquoted to another tube, it should be paid attention not to take bottom residual platelets. A single freeze-thaw procedure causes disintegration of platelets in plasma samples separated by centrifugation. This disintegration causes an increase in analytes such as plasminogen activator inhibitor-1 found particularly in platelets. Besides, anionic phospholipids found in residual platelet membranes affect phospholipid-based coagulation tests. Phospholipids releasing from platelets during freezing-thawing of the sample can mask certain lupus anticoagulants and lead to pseudo-negative results.

Recommendation:

- If coagulation tests are not immediately studied but stored, it should be paid attention to not taking bottom platelet residues while aliquoting the sample.

8. SAMPLE STORAGE

Allowed period between sampling for coagulation tests and analyses depends on tests to be performed and temperature during both handling and storage of the sample. If citrated whole blood sample shall not be used within a short period of time (<30 minutes) after sampling, its cap should be kept closed (4). Sample for coagulation testing should be processed as soon as possible and kept under appropriate conditions. For these tests, conditions of a short and long period of sample storage vary. Laboratories can specify their own sample storage conditions, but these conditions should be tested and verified.

8.1. Short-Term Storage Until Analysis

Various information is available in the CLSI H21 A5 guideline and other stability studies on the stability of different coagulation tests in the continuum from sampling to centrifugation in the citrated whole blood specimen (Table 2).

	Stability of whole blood sample	
Test name	**CLSI** (4)	**Other resources (50-55)**
PZ	24 hours	24-72 hours
aPTZ	4 hours	18-24 hours
Fibrinogen	4 hours	48 hours- 7 gün
D-dimer	4 hours	48 hours
Factor II, VII, IX, X and XI	4 hours	48 hours
Factor V and VIII	4 hours	24 hours
von Willebrand factor antigen, and von Willebrand factor ristocetin cofactor	4 hours	24-48 hours
Antithrombin activity	4 hours	48 hours-7 gün
Protein C activity	4 hours	48 hours
Protein S activity	4 hours	4-6 hours
Free protein S	4 hours	24 hours
aPTT or anti-Xa in unfractionated heparin containing sample	1 hour	-
aPTT or anti-Xa in low molecular weight heparin containing sample	4 hours	24 hours

Table 2. Stability of whole blood sample in coagulation tests

Blood sample should be kept capped, in the upright position and at room temperature (37). It is not recommended to keep it refrigerated (2-8oC) since cold causes FVII activation and FVIII loss. Accordingly, PT and aPTT test results are affected (37,41).

For PT and D-dimer testing in samples centrifuged or non-centrifuged after being collected, although this period prolongs up to 24 hours in publications, it is important to control this period for laboratories with respect to their own conditions, because sample stability is related to blood collection tube, coagulometer, thromboplastin reactive or their composition. One must be careful about whole blood samples kept without centrifugation at room temperature, because this can cause mechanical agitation, pseudo-high values by an unknown mechanism in PT/INR tests (4,38). It is not recommended to keep in refrigerator (2-8oC) because cold causes FVII activation and variations in PT results. If the patient is receiving concomitant heparin and coumarin anticoagulation treatment, and if reactive used for PT does not contain heparin neutralizing agent, PT can vary with the time of sample storage (4).

For patients who don't receive standard (unfractionated) heparin teraphy, samples for aPTT and specific coagulation tests (such as factor measurements, LA and VWF measurement) can be stored at room temperature for 4 hours non-centrifuged or centrifuged and with its plasma, and capped (48,50). If aPTT analysis exceeds 4 hours, it should be validated with respect to the effects of this period on test results in patient serial including normal and pathological aPTT samples. In the same validation study, FV and FVIII activities must also be studied. It is crucial to separate plasma in another tube after the centrifugation of blood sample in order to potential neutralization of heparin if aPTT test is ordered for heparin treatment follow up (4,38). In the follow-up of patients using standard heparin, samples collected for aPTT test should be waited at room temperature, centrifuged within 1 hour and studies within 4 hours.

Recommendation:

- Coagulation tube should be kept capped, in upright position and at room temperature (18-25oC).
- All coagulation measurements should be performed within the first 4 hours following sampling.
- PT and D-dimer test can be studied within 24 hours. But laboratories should evaluate sample stability with respect to their own systems.
- If aPTT test is ordered for standard heparin monitoring, whole blood sample should be centrifuged, and plasma should be separated within the first hour after sampling.

8.2. Long-Term Storage Until Analysis

If the samples cannot be studied for PT and D-dimer in a period shorter than 24 hours and aPTT and for routine and specific coagulation tests in a period shorter than 4 hours, plasma samples should be separated without contamination of residual bottom cells, aliquoted and stored frozen (4). If the samples will be studied within 2 weeks, they can be stored at -20oC freezer. However, the freezer must be free of automatic freeze-thaw feature, because these automatic freeze-thaw cycles can cause activation of FVII with cold and degradation of other factors (4). Longer storage (longer than 2 weeks) should be done at -70oC or in colder freezers (51).

Recommendation:

- If test analysis will exceed above mentioned periods, in order to keep the plasma sample for <2 weeks, storage should be at -20oC and for longer periods at <-70oC.

8.3. Freeze-Thaw Procedures

Overall, it is recommended to use coagulation test sample through only one freeze-thaw process. It should be avoided more than one freeze-thaw cycle. However, it is stated that FII, FVII, FX, FIX, FXI activities and ATIII, protein C, VWF and plasminogen are not affected by multi freeze-thaw cycles (37).

Recommendation:

- Each laboratory should specify its own sample number that it will portion for specific coagulation tests and analysis time according to its own conditions.

8.4. Thawing Frozen Plasma Samples

Frozen plasma samples should be thawed in incubator, hot dry block or water bath at 37oC for 5-10 minutes (3,4,37). Thawed samples should be mixed sufficiently in order to ensure sample integrity prior to the analytical phase. The most appropriate mixing pattern is to turn upside down with an 180o angle and doing this 6 times. If samples are not thawed completely or are waited for a long time at 37oC, change in coagulation factor activities and impaired sample integrity lead to erroneous test results (3,37,52). If water bath is used in thawing process, it should be paid attention not to impair the integrity of the barcode bearing patient's information.

Recommendation:

- Frozen plasma samples for coagulation tests should be thawed in hot dry block or water bath at 37oC for 5-10 minutes.
- Samples should be mixed sufficiently by turning upside down before analyses.

9. HEMOLYSIS, ICTERUS, AND LIPEMIA INTERFERENCE IN COAGULATION TESTS

Hemolysis, icterus and lipemia interfere light transmittance according to plasma content in whole blood, test principle and device features and cause variations in test results (4).

9.1. Hemolysis

Hemolysis is a state developed by free hemoglobin and erythrocyte lysis products arising from fractionated erythrocyte content in whole blood. It can be assessed as the level of free hemoglobin in plasma increases over 0.2 g/L (52). Hemolysis can occur in vivo conditions which are hereditary, acquired or iatrogenic clinical states causing erythrocyte lysis (such as hemolytic anemia, severe infections, disseminated intravascular coagulation, transfusion reactions) and in vitro conditions in stages of blood collection and its subsequent stages. Usually it is evaluated in vitro and used as an indicator for monitoring sample quality (53). Every coagulation test extremely depends on the quality of plasma obtained (13).

With hemolysis, release of erythrocyte or membrane components (phospholipids, enzymes, proteins, ADP, etc.) activates or inhibits in vitro primary and secondary hemostasis. In addition, free hemoglobin can create interference spectrophotometrically or due to its pseudo-peroxidase activity. The Guidelines of the Clinical & Laboratory Standards Institute (CLSI) recommends not studying the samples with visible hemolysis due to potential activation of coagulation factors and end-point measurement interferences (13). There exists more than one theory for interference created by hemolysis. It can be suggested that hemolysis provides a tissue factor source that can cause activation of coagulation factors and shortening the results. And another theory is that hemolysis process competes with coagulation reagents thus leads to prolongation of coagulation test results (52). These interferences, if it is used whether photo optical or electromechanical end-point measurement method, can rise variations in coagulation tests in wide range. That is to say, results can be improperly high or low due to dilutional effects. The following effects are clearly observed with hemolysis:

- Decrease in fibrinogen levels with elevation of hemolysis,
- Increase in D-dimer level,
- Decrease in anti-thrombin level (38, 52).

In literature, it is reported that evaluation of the results of PT and aPTT tests performed in samples with hemolysis display variations (3,13,39). It cannot be assumed that coagulation test values prolong due to hemolysis (52). Comparing different studies conducted with different combinations of test methods, reagents and devices is very difficult. Besides, in interference studies conducted with hemolysate, it is discussed that differences in hemolysate formation techniques are also effective on results and this in turn complicates interpretation. Therefore, local evaluation of test principles and device features and prefiguration about interferences are advised to laboratories (Figure 4) (54).

Figure 4. Examples of optic and photometric coagulation devices used in laboratories.

Excessively hemolyzed samples should not be used due to potential interference and must be re-sampled. Free hemoglobin amount in different devices corresponds to different hemolysis index level. In cases where hemolysis index is integrated to the system, test/device manufacturer's cut-off values related to

hemolysis interference should be known (52). Plasma samples that hemolysis can be visually detected (pink-red color) should not be used in analyzers which measures with an optic system due to interference with light transmittance. Use of mechanical end-point measurement method is ideal instead of the rejection of hemolyzed plasma. Coagulation mechanism activated as a result of thromboplastin-like procoagulant release following endothelial damage can alter sample quality. This will be effective in both photo-optical and electromechanical devices, because though its analytical effects are small, biological effects are important. Because of this opinion, it is also advised not to report the results of hemolyzed sample (54).

9.2. Icterus

Hyperbilirubinemia being defined as >1.5 mg/dL bilirubin concentration creates interference originated from frequency aliasing in coagulation tests. Bilirubin has a high absorption spectrum between 400-520 nm (54). When it is measured by an optic system using high wavelengths more than 570 nm, it is found out that test results of icteric samples are accordant with electromagnetic method (55). On the condition that a second different wavelength (650 nm and over) is selected in icteric samples, if bilirubin concentration is <20 mg/dL, interference is not expected in coagulation test results (54).

9.3. Lipemia

In lipemic samples, turbidity is essentially originated from large lipid particles and lipid concentration is usually over 500 mg/dL. Lipemia can be observed in samples collected in postprandial state, administration of intravenous lipids, disorders such as diabetes, chronic alcohol consumption, impaired renal function, thyroid disorders, acute pancreatitis, myeloma, primary biliary cirrhosis, systemic lupus erythematosus and in drug utilizations such as estrogen, steroids, protease inhibitors (54). Interference observed in lipemic samples is both optic- and biologic-origin. Biological effects develop from different sources. Acute increase in FVII activity is observed following greasy food and frequently associated with an increase in FVIIa concentration. Fatty meals cause decreases in the activity of some coagulation factors (such as FII, FIX, FX, FVII, FVIIa, FXIIa) with its acute effect on platelet function (3). It is demonstrated that FIX activity in lipemia period increases following fatty diet, and thromboplastin time varies with differences in meal substance (low-fat, foods with herbal-oil) (56). In a study researching platelet and monocyte activation of lipemia after a fatty meal, using monoclonal antibodies, it is demonstrated that percentage of platelets expressing surface P-selectin and the activated conformation the GPIIb-IIa receptor is high (57). Significant lipemia can create interference by impairing optic absorbance or light transmittance or

due to light scattering particularly in short wavelengths below 500 nm. This interference can be prevented by using a special wavelength (650 nm and over). Analytical interference occurs in tests especially working based on detection of optic clot. In order to minimize this effect, it is recommended to use devices working based on electromechanics or devices performing coagulation tests in alternative wavelengths. However, unless a metabolic disorder such as dyslipidemia exists, the best approach is to repeat sample collection in fasting state (3). Although different methods can be used for removing lipids for decreasing lipemia interference (ultracentrifugation, lipid cleaning chemical applications or dilution), no single approach is yet could be recommended for reliable results. Ultracentrifugation can result in precipitation of large protein masses such as fibrinogen or FVII/vWF complex thus can lead to erroneous results. Organic solvents such as n-hexane can be used for removing lipids, but it should be verified if measured analyte is affected or not. If it is measured turbidimetrically like quantitative D-dimer and vWF activity/antigen, effect if lipemia can be decreased by plasma dilution. Naturally, dilution method cannot be used for PT, aPTT tests (3, 53).

Recommendation:

- Laboratories should determine affection level of their coagulation tests by hemolysis, lipemia and icterus on their own terms.
- Samples must be evaluated with respect to these interferences before analysis.

10. FACTORS INFLUENCING COAGULATION TESTS RESULTS

10.1. Circadian Rhythm

Platelets which are one of the most important elements of coagulation system maximize numerically in the afternoon and as activity in the morning (58). It is documented by studies that both aPTT and PT tests are measured short in the morning. (Within 24 hours, the difference between the longest and the shortest time is calculated to be 0.95 seconds for PT and 3.27 seconds for aPTT. However, it is indicated that this difference is not clinically significant) (59). It is demonstrated by studies that endogen coagulation inhibitors protein S and C along with ATIII reach their highest concentrations in the morning hours (6:00) and their lowest concentrations at noon (58). And fibrinogen is measured in high concentrations in the morning depending on the circadian rhythm of interleukin 6 (in high concentrations nocturnally) which stimulates its synthesis (60).

10.2. Posture

Significant effect of the posture variations on routine coagulation tests used in both diagnosis and follow-up is shown by studies (61). This variation is particularly effective while the patient is moving from supine position to sitting position or standing, then the variation is for fibrinogen and PT towards decreasing and for aPTT towards increasing. 3.7% decrease in PT obtained in patients moving from supine position to standing can cause unnecessary drug dose changes particularly in patients who are receiving vitamin K. Fibrinogen decrease can be explained with plasma escape occurring while standing up in upright position for a long time, diffusion of filterable elements and water to interstitial space, inclosing plasma components which are larger and not exposed to diffusion (62). However, it is discussed that plasma escape and hemoconcentration occurring during moving from horizontal position to sitting or standing position activates extrinsic pathway in blood coagulation and causes PT shortenings (61).

10.3. Daily Diet and Smoking

There is no satisfactory evidence in the literature about the effects of daily diet (nocturnal nutrition, excessive fatty food, consumption of caffeinated beverages) and smoking on coagulation tests. However, it is known that lipemic samples display interference in several tests (63).

Abstaining from smoking for 2 h prior to the venipuncture is recommended, because of a potential effect on platelet aggregation (63).

10.4. Physical Activity

Coagulation and fibrinolysis measurements vary depending on the intensity and duration of exercise. This effect is based on the individual's age and physical condition (64,65). While aPTT decreases during heavy resistant exercise such as maximal weight strength exercises (like weightlifting), marathon and body building, it is not observed any variation in PT (66). It is considered that this decrease in aPTT is due to an increase in FVIII. FVIII, vWF antigen and vWF ristosetin cofactor activities increases 2.5-fold with exercise and this increase lasts for about 10 hours after the end of exercise. It is not observed significant variations in FXII, FV, FVII, FII and fibrinogen levels (67). Thrombin level increases within 30 minutes with moderate exercise, but this increase remains in reference range even with heavy exercise. Increases in D-dimer level are also seen and this increase lasts for about an hour (68).

10.5. Menstrual Cycle and Hormone Replacement Therapy (HRT)

In hemostatic variables such as FII, FVII, FX, ATIII, APCR, plasminogen and D-dimer, very little variability is observed throughout menstrual cycle (69-71). However, variations observed in menstrual cycle occur most frequently in luteal phase. In follicular phase, while FVII and FVIIa levels increase, protein-S level decreases. In luteal phase, while FVIII, vWF antigen and ristosetin cofactor levels and fibrinogen level increase (63).

HRT effects based on the estrogen component of the treatment. There is no clear information about the effect of progesterone alone. The grade of classical APCR which is induced by combined oral contraceptives and other hemostatic variances is determined by progestins (72). In healthy menopausal women, an increase is determined in coagulation factors such as FVIII and fibrinogen. These effects are related to age and estrogen levels (73). It is reported that, in menopausal women, initiating replacement therapy activates coagulation system by increasing FI and FII and decreasing APCR and protein S levels (74,75). Variations in coagulation processes is less for transdermal HRT therapy when compared to oral HRT therapy (76).

10.6. Pregnancy and Coagulation

Complex hemostasis process complicates more due to physiological alterations resulting from prevention of bleeding during childbirth in pregnancy. During pregnancy, multiple alterations occurs in coagulation system (77). Decreases in platelets and coagulation factors are resulting from uteroplacental consumption and dilutional effects. This decrease becomes more evident due to increasing in degradation and hemodilution especially in the 3rd trimester. Despite these variations, variation is not observed in the results of routine coagulation tests (PT and PTT) or a slight decrease is observed. In pregnancy, fibrinolysis process significantly decreases. Fibrinogen level increases up to 200% in reference to pre-pregnancy. Plasminogen activator inhibitor 1 and 2 (PAI-1 and 2) releasing from placenta increase and the level of tissue plasminogen activator decreases. In addition, thrombin-activatable fibrinolysis inhibitor level increases significantly in the 3rd trimester. D-dimer level increases in pregnancy, but this increase does not result from intravascular coagulation increment (78). Variation in coagulation system in pregnancy is shown in Table 3.

Hemostasis parameters	Changes in Pregnancy
FII and FV	Generally stable
FVII	1000% increase
FVIII, FIX, FXII, VWF, RCOA (ristocetin cofactor)	More than 100% increase
FXI	Variable (stable or increase)
FXIII	50% increase
Protein- C	Generally stable
Protein-S	50% decrease
Fibrinogen	More than 100% increase
D-Dimer	400% increase
t-PA activity	Decrease
PAI-1 and 2	Increase
Platelet count	20% decrease

Table 3. Changes in coagulation tests monitored in pregnancy.

Recommendations:

- Intensive physical exercise should be avoided 24 hours before sampling.
- 8-12 hours fasting is recommended before sampling.
- It is recommended not to smoke at least 2 hours before sampling for coagulation tests.
- In women who are receiving combined oral contraceptive and HRT, these treatments should be discontinued two months before protein-S, protein-C and APCR measurements.
- Sampling is required for hereditary disorders like vWF, deficiency of protein S, sampling should be performed when normal menstrual cycle begins or at 2 months after giving birth. All abnormal values including pregnancy related antiphospholipid antibodies should be confirmed by repeating blood collection.

10.7. Drug, Nutritional Supplement, Herbal Remedy Interactions

The necessity to inform laboratory specialist about the drugs received by the patient during evaluating coagulation test results is stated in articles 3. and 4.1 of this guideline. Potential effects of drugs, nutritional supplements and herbal remedies used by individuals on routine biochemical tests are listed in Table 4.

Agents	PT/INR (sensi-tive to FI, FII, FV, FVII, FX deficiency and can be used in re-place-ment moni-toring) (79)	aPTT (sensi-tive to FI, FII, FV, FVIII, FIX, FX, FXI, FXII deficiency and can be used in re-place-ment monitor-ing) (79)	Fibri-nogen	Explanation
Warfarin (VKA) (80) (81)	↑	Mild prolonga-tion		INR is used in drug level monitoring (Increase in PT rises with azitromy-cin, ciprofloxacin, clar-ithromycin, flucona-zole and other azol anti-fungal agents, levofloxacin, metro-nidazole, trime-thoprim-sul-famethoxazole)
Heparin (LMWH) (80)	-	↑ low sensitivity		Can be used along with prothrombin time prolongation. Unsatisfactory in drug level moni-tor-ing.
Heparin (UFH) (80)	-/mild prolongation	↑ high sensitivity		Prolongation in PT, thrombin time very sensitive. Used in drug level monitor-ing with anti-FXa.
Coumarin (80)	↑	↑		INR is used in drug level monitoring

Apixaban (Factor 10a inhibitor) DOAC (81, 82)	variable	↑		In PT, device and reagent dependent drug level monitoring, antiFXa level is used.
Dabigatran (Thrombin inhibitor) DOAC (83)	-	↑ mild, not linear		Used in Anti FIIa drug level monitoring (80). Thrombin time and fibrinogen elevation together can be a reliable monitorization method (83).
Rivaroxaban (Factor 10a inhibitor) DOAC (80)	↑ (in some reagents)	↑ dose dependent, less sensitive than PT		Anti-FXa is used in drug level monitorization
Amikacin (84)	–	↑	–	No change in thrombin time
Gentamicin (84)	–	↑	–	No change in thrombin time
Daptomicin (85)	↑			Antibiotic from the cyclic lipopeptide class
Tigecycline (86)	↑	↑	↓	Broad-spectrum antibiotic (iv). Prolongation in thrombin time.
Antibiotics containing N-methyl-tio-tetrazole side chain (87, 88)	↑			2nd and 3rd generation cephalosporins (Moxalactam, cephaperazone)
Oritavancin (89)	↑	↑		Lipoglycopeptide antibiotic Prolongation in phospholipid containing reagents is monitored dose dependent

Teicoplanin (90)	–	–		
Metformin (91)	–	–	-/mild ↓	Fibrinogen depression is explicit in combination with fenofibrate (92).
In PCOS treatment/antiandrogenic OKS+metformin (93)	↓/↑	–/↓	↑	
Glucocorticoid (94)			Low dose ↓/ high dose ↑	Doze dependent biphasic response
Argininemia (urea cycle disorder) (95)	↑	↑		Low FVII and FIX, normal FII and FX in prognosis
Fish oil (PUFA) (96)	-	-		For use of 1,5g/day eicosapentaenoic acid+ docosahexaenoic acid (EPA-DHA) for 52 weeks (8), EPA in men and DHA in women decrease platelet aggregation more effectively (11).
L-carnitine nutritional supplement (97)			↓	No change in FV, FVII, FIX and protein C activity after using 1000 mg/day for 12 weeks

Nutritional supplements (98)	No clinical trial			There are some data suggesting that garlic, echinacea, ginger, green tea, fish oil which are known to effect platelet function and coagulation increase the risk of bleeding (11).
Herbal remedy+warfarin interaction (99)*	↑			Observed in combination of wolf berry (Lyciumbarbarum)/ quilinggao/ boldo-caraway (boldo/fenugreek)
Valproic acid (100)			↓	Within 6 months following the initiation of treatment

Table 4: Interfernces that can occur due to drug, nutritional supplements and herbal remedy interactions

DOAK: direct oral anticoagulants **VKA**: Vitamin K antagonists **OKS**: Oral contraceptives

*Herbal products which are considered to interact with warfarin in some case reports: Astragalus, caraway (boldo/fenugreek), green jam chlorella (chlorella), chondroitin sulphate, melatonin, co-enzyme Q10, papain, cornelian cherry, bilberry, alligator pear, ginseng, gingkoblabo, St. John's Wort, red sage, grapefruit juice, green tea, wolf grape (lyciumbarbarum), soya, scrub palmetto extract (palmetto/ serenoarepens) (99).

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